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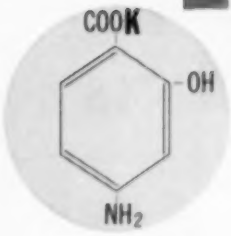

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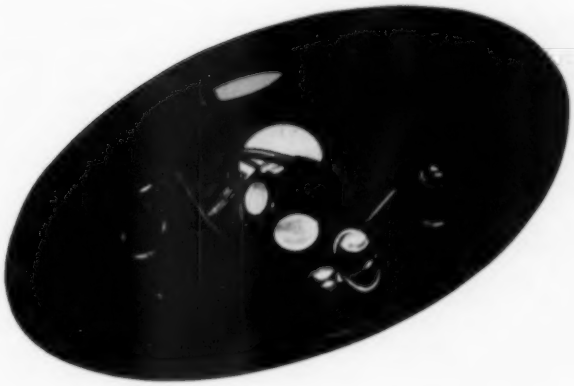

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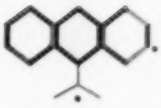

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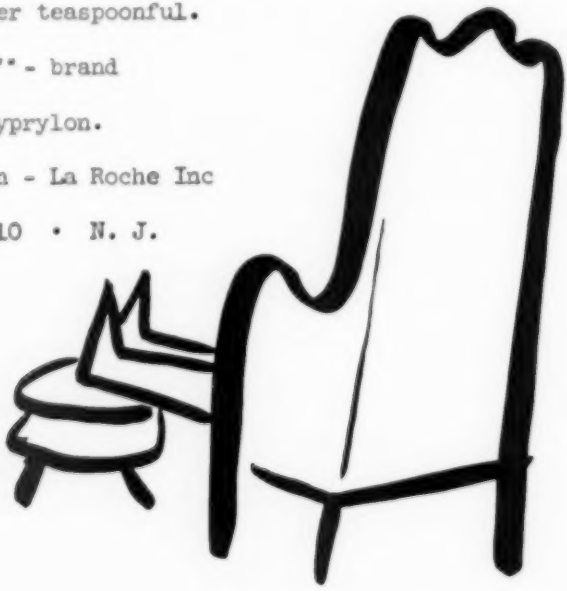

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THE PREVALENCE OF STREPTOMYCIN- AND ISONIAZID-RESISTANT STRAINS OF MYCOBACTERIUM TUBERCULOSIS IN PATIENTS WITH NEWLY DISCOVERED AND UNTREATED ACTIVE PULMONARY TUBERCULOSIS[1]

A. D. CHAVES, A. B. ROBINS, H. ABELES, L. R. PEIZER, G. DANGLER, AND D. WIDELOCK

(Received for publication April 8, 1955)

INTRODUCTION

Between July, 1953, and January, 1955, the Bureaus of Tuberculosis and Laboratories, New York City Department of Health, carried out a study to determine the frequency with which streptomycin- and isoniazid-resistant strains of *Mycobacterium tuberculosis* could be demonstrated in the sputum of patients with newly discovered and as yet untreated active pulmonary tuberculosis.

PLAN OF STUDY

Selection of Material

The professional personnel of all twenty-three chest clinics of the Bureau of Tuberculosis were instructed to send to the central laboratory two specially identified twenty-four-hour sputum specimens[2] for all patients presumed to have recently developed active pulmonary tuberculosis. The records of those patients for whom such specimens were received were then reviewed by one of the present writers for accuracy of diagnosis, history of exposure to tuberculosis, duration of disease, and the type of treatment[3] received. All patients who were registered as having active pulmonary tuberculosis and who, as far as could be determined, had never received antimicrobial treatment for this disease were admitted to this study if they could be classified into one of the following two categories:

> *Newly reported case of active pulmonary tuberculosis:* A patient whose sputum specimen was received in the laboratory within a period of six months following the date when the diagnosis of active pulmonary tuberculosis was made for the first time.

[1] From the Bureaus of Tuberculosis and Laboratories, Department of Health, 125 Worth Street, New York 13, New York.

[2] In many instances, only one satisfactory specimen was received.

[3] For the purpose of this report, all references to treatment will apply only to antimicrobial treatment (streptomycin or isoniazid).

Recently reactivated case of pulmonary tuberculosis: A patient whose sputum specimen was received in the laboratory within a period of six months following the date when the diagnosis of a recent reactivation was made, and who was known to have had arrested or inactive pulmonary tuberculosis for a minimal period of one year prior to the reactivation.

No attempt was made to correlate the clinical response of any of the patients to antimicrobial therapy with the degree of drug resistance of their organisms.

All of the patients included in this study were adults; a more detailed analysis of the age distribution of this series, however, was not made.

Laboratory Methods

In most instances, the sputum was digested by the trisodium phosphate transport-digestion method previously described (1). After digestion, an equal amount (0.25 ml.) of original sputum concentrate was inoculated both on drugless media (control) and drug-containing media, using the techniques which have already been reported (2, 3). For determining susceptibility to streptomycin, slants incorporating 10 γ, 100 γ, and 1,000 γ of the drug per ml. of medium were used; for isoniazid, slants incorporating 0.1 γ, 1.0 γ, and 5.0 γ of the drug per ml. of medium were employed.

Culture and degrees of drug resistance were read in the manner previously noted (2, 3), using the following semiquantitative estimate of growth:

4-plus = confluent growth
3-plus = heavy, discrete growth, more than 150 colonies
2-plus = 100 to 150 colonies
1-plus = 50 to 100 colonies
less than 1-plus = less than 50 colonies, number of colonies reported as found

RESULTS

Eight hundred and ninety-eight patients were included in the study after screening a much larger number and eliminating those who did not fulfill the criteria for selection or who submitted inadequate sputum specimens. Of this group, 610 represented newly diagnosed and reported cases of active pulmonary tuberculosis and represented less than 30 per cent[4] of such cases registered from the chest clinics during the period of investigation. The 288 cases of recently reactivated disease were selected from an undetermined number of such cases seen in the clinics during the period of study.

The cultural findings of the sputum for the entire group of patients are presented in table 1. *M. tuberculosis* was demonstrated by culture of the specimens of 385 patients (43 per cent). *Some growth of M. tuberculosis on drug-containing medium was observed in 43 instances, or in 11 per cent of the patients with positive cultures.* Instances of growth of tubercle bacilli in drug medium were slightly more frequent among the newly reported than among the recently reactivated cases.

[4] This relatively low percentage was due to: (*1*) administrative failure of personnel to comply with instructions; (*2*) failure of patients to submit specimens as requested; (*3*) contamination or leakage of specimens submitted. Because the cases were accepted into the study at random, it was believed that these 898 patients were a representative sample of the entire group of newly diagnosed cases registered from the clinics during the period of study.

TABLE 1

CULTURAL FINDINGS ON DRUGLESS AND DRUG-CONTAINING MEDIA INOCULATED WITH SPUTUM SPECIMENS FROM UNTREATED NEWLY REPORTED ACTIVE AND NEWLY REACTIVATED CASES OF PULMONARY TUBERCULOSIS

Department of Health, the City of New York

July 1, 1953 to January 1, 1955

Cultural Findings	Type of Case		Total		
	Newly Reported	Recently Reactivated	Number	Per Cent	
Total	610	288	898	100.0	
Negative on drugless medium (control)	325	188	513	57.0	
Positive on drugless medium (control)	285	100	385	43.0	100.0
No growth on any drug-containing media	246	96	342	88.8	
Some growth on drug-containing media total	39	4	43	11.2	
(*a*) Streptomycin alone	16	3	19		4.9
(*b*) Isoniazid alone	13	1	14		3.6
(*c*) Streptomycin and isoniazid	10	0	10		2.6
(*a*) + (*c*) Streptomycin—total	26	3	29	7.5	
(*b*) + (*c*) Isoniazid—total	23	1	24	6.2	

TABLE 2

QUANTITATIVE FINDINGS ON DRUGLESS MEDIUM OF CULTURES OF TUBERCLE BACILLI FROM 385 UNTREATED PATIENTS WITH NEWLY DISCOVERED ACTIVE PULMONARY TUBERCULOSIS BY ABSENCE OR PRESENCE OF ANY GROWTH ON DRUG-CONTAINING MEDIA

Growth on Drug-Containing Media	Total		Quantitative Findings on Drugless Medium (Control)					
			4+ and 3+		2+ and 1+		Less than 1+	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	385	100.0	134	100.0	145	100.0	106	100.0
Newly reported	*285*		*117*		*103*		*65*	
Recently reactivated	*100*		*17*		*42*		*41*	
No growth on either streptomycin or isoniazid-containing media	342	88.8	113	84.4	130	89.6	99	93.4
Some growth on either streptomycin or isoniazid-containing media	43	11.2	21	15.6	15	10.4	7	6.6
Streptomycin alone	19		7		7		5	
Isoniazid alone	14		8		5		1	
Both streptomycin and isoniazid	10		6		3		1	

TABLE 3

DETAILED ANALYSIS OF CULTURAL FINDINGS OF 43 PATIENTS WITH RECENTLY DISCOVERED AND UNTREATED ACTIVE TUBERCULOSIS FROM WHOM STRAINS OF M. TUBERCULOSIS WERE ISOLATED WHICH SHOWED ANY GROWTH ON DRUG CONTAINING MEDIUM

Case Number and Initials	Month and Year of Diagnosis	Drugless Medium (Control)	Amount of Streptomycin Incorporated into Medium in γ per Ml.			Amount of Isoniazid Incorporated into Medium in γ per Ml.		
			10.0	100.0	1,000.0	0.1	1.0	5.0
Newly Reported Cases								
Some resistance to streptomycin alone								
1. A. V.	7/53	3+	3+	3+	3+			
2. J. R.	2/54	4+	4+	4+	0			
3. F. B.	2/54	4+	4+	4+	0			
4. H. F.	2/54	1+	1+	10 colonies	0			
5. A. G.	1/54	1+	1+	4 colonies	0			
6. D. R.	9/54	4+	4+	0	0			
7. S. N.	2/54	3+	3+	0	0			
8. G. F.	8/53	2+	2+	0	0			
9. A. Mu.	1/54	1+	1+	0	0			
10. M. T.	2/54	1+	1+	0	0			
11. L. J.	7/53	4+	2+	0	0			
12. D. K.	11/53	4+	1+	0	0			
13. M. Dr.	5/53	2+	5 colonies	0	0			
14. M. F.	7/53	20 colonies	10 colonies	1+	0			
15. E. B.	6/53	20 colonies	1 colony	0	0			
16. J. C.	12/53	20 colonies	1 colony	0	0			
Some resistance to isoniazid alone								
17. A. H.	6/54	4+				3+	3+	3+
18. J. So.	6/54	3+				3+	3+	3+
19. E. B.	6/54	2+				2+	2+	2+
20. A. J.	9/53	1+				1+	1+	1+
21. P. F.	9/53	2+				1+	13 colonies	5 colonies
22. U. D.	6/54	1+				1+	1+	2 colonies
23. U. Ra.	11/54	4+				4+	0	0
24. L. B.	8/54	2+				2+	0	0
25. B. L.	3/54	1 colony				1 colony	0	0
26. E. H.	7/53	4+				2+	0	0
27. H. O.	6/53	4+				1+	0	0
28. N. C.	11/53	4+				12 colonies	0	0
29. J. Nu.	9/53	4+				6 colonies	0	0
Some resistance to both streptomycin and isoniazid								
30. M. G.	6/53	4+	1 colony	0	0	5 colonies	1 colony	1 colony
31. W. R.	2/54	3 colonies	1 colony	0	0	3 colonies	2 colonies	0
32. W. S.	6/53	4+	1+	0	0	1+	0	0
33. L. B.	7/53	4+	15 colonies	0	0	5 colonies	0	0
34. J. T.	6/53	4+	1 colony	0	0	1+	0	0
35. M. L.	3/53	4+	6 colonies	0	0	1+	0	0
36. E. L.	10/53	1+	0	3 colonies	3 colonies	1 colony	0	0
37. J. G.	6/53	2+	2 colonies	0	0	25 colonies	0	0
38. R. F.	8/53	3+	4 colonies	0	0	1+	0	0
39. J. S.	2/54	1+	5 colonies	0	0	30 colonies	0	0
Recently Reactivated Cases								
Some resistance to streptomycin alone								
40. W. T.	2/54	8 colonies	4 colonies	6 colonies	0			
41. J. K.	2/54	3 colonies	2 colonies	0	0			
42. C. J.	3/54	2+	6 colonies	0	0			
Some resistance to isoniazid alone								
43. L. K.	8/54	4+				3+	3+	3+

TABLE 4

DISTRIBUTION OF CULTURES POSITIVE FOR TUBERCLE BACILLI FROM 385 UNTREATED PATIENTS ACCORDING TO DEGREE OF RESISTANCE SHOWN BY THE ISOLATED STRAINS OF *M. TUBERCULOSIS* TO STREPTOMYCIN AND ISONIAZID AS DETERMINED BY THE HIGHEST CONCENTRATION OF DRUG PER MILLILITER OF MEDIUM ON WHICH THERE WAS ANY GROWTH

Type of Case	Total Positive Cultures	Streptomycin Resistance					Isoniazid Resistance				
		No Growth on Any Streptomycin-Containing Medium	Some Growth on Streptomycin-Containing Medium				No Growth on Any Isoniazid-Containing Medium	Some Growth on Isoniazid-Containing Medium			
			Highest Concentration in γ per Ml. of Medium on Which Any Growth Was Seen			Total		Highest Concentration in γ per Ml. of Medium on Which Any Growth Was Seen			Total
			10	100	1,000			0.1	1.0	5.0	
Newly reported	285	259	19	5	2	26	262	15	1	7	23
Recently reactivated	100	97	2	1	0	3	99	0	0	1	1
Total	385	356	21	6	2	29	361	15	1	8	24

The semiquantitative findings on drugless medium (control) for the entire group of 385 sputum cultures[5] are shown in table 2. Some degree of growth on drug-containing medium was noted in 15.6 per cent of the specimens which showed 3-plus or 4-plus growth in the control tube. The specimens with less than 1-plus growth in the control tube showed growth of tubercle bacilli in 6.6 per cent when cultured on the drug-containing medium. This difference is of borderline statistical significance. In general, there was much heavier growth of tubercle bacilli from the sputum obtained from newly reported than from recently reactivated cases. This, in part, may account for the proportionately smaller numbers of specimens from the latter group which showed growth on drug-containing media (table 1).

In table 3, the cultural findings are listed in detail, both for drugless and drug-containing media, for the 43 specimens from which strains of *M. tuberculosis* were isolated which showed any degree of growth on a drug-containing medium.

Of the 385 positive specimens, 29 (7.5 per cent) contained strains of *M. tuberculosis* which showed some growth on streptomycin-containing medium and 24 (6.2 per cent) contained strains which showed some growth on isoniazid-containing medium (*see* table 1). In table 4 is indicated the distribution of all the sputum specimens positive for tubercle bacilli, according to the degree of resistance shown to streptomycin and isoniazid as measured by the highest concentration of the drug which permitted any growth.

The number and percentage of patients whose sputum contained strains of *M. tuberculosis* displaying significant resistance to streptomycin and isoniazid

[5] When both specimens from a single patient were positive for *M. tuberculosis*, only the culture with the heavier growth was evaluated.

TABLE 5

PROPORTION OF 385 UNTREATED PATIENTS WITH RECENTLY DISCOVERED ACTIVE TUBERCULOSIS WHOSE ORGANISMS SHOWED SIGNIFICANT RESISTANCE* TO STREPTOMYCIN OR ISONIAZID

Drug† to Which Significant Resistance Was Demonstrated	Quantitative Findings on Control Tube						Total‡ (385 Patients)	
	4+ and 3+ (134 Patients)		2+ and 1+ (145 Patients)		Less than 1+ (106 Patients)			
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Streptomycin	3	2.2	1	0.7	2	1.9	6	1.6
Isoniazid	4	3.0	4	2.8	1	0.9	9	2.3

* Significant resistance to streptomycin: Any growth on 1,000.0 γ of streptomycin per ml. of medium or growth on 100.0 γ of streptomycin per ml. equivalent to that on the control tube.

Significant resistance to isoniazid: Any growth on 5.0 γ of isoniazid per ml. of medium or growth on 1.0 γ of isoniazid per ml. equivalent to that on the control tube.

† No patient had organisms which were significantly resistant to both isoniazid and streptomycin.

‡ Of 285 newly reported patients (exclusive of 100 with recently reactivated disease), 5 (1.8 per cent) had tubercle bacilli which were significantly resistant to streptomycin and 8 (2.8 per cent) had tubercle bacilli which were significantly resistant to isoniazid.

are shown in table 5. For the purpose of this report, significant drug resistance was defined as follows:

Significant streptomycin resistance: Any growth on 1,000 γ of streptomycin per ml. of medium or growth on 100 γ of streptomycin equivalent to that on the control.

Significant isoniazid resistance: Any growth on 5.0 γ of isoniazid per ml. of medium or growth on 1.0 γ of isoniazid equivalent to that on the control.

Using these criteria, a total of 6 patients (1.6 per cent) of those with positive cultures had tubercle bacilli significantly resistant to streptomycin, and 9 patients (2.3 per cent) had organisms significantly resistant to isoniazid. Among the 134 patients whose specimens yielded a 3-plus or 4-plus growth on the drug-less medium, 3 (2.2 per cent) had organisms significantly resistant to streptomycin and 4 (3.0 per cent) had organisms significantly resistant to isoniazid. In no instance was a strain of *M. tuberculosis* isolated which was significantly resistant to both drugs.

Among the 43 patients with strains of tubercle bacilli which showed any growth on drug-containing medium, there were 5 with a history of exposure to a previously treated case of active tuberculosis. Accurate details concerning the type of treatment given to these possible source cases could not be obtained.

DISCUSSION

Even though streptomycin has now been used in the treatment of tuberculosis for almost ten years, it was encouraging to find that only 1.6 per cent of the strains of *M. tuberculosis* isolated from untreated patients were significantly resistant to streptomycin *in vitro*. This finding is essentially in agreement with

that of Cummings and Livings among recently hospitalized and previously untreated patients (4).

With isoniazid, too, the results were encouraging. In spite of widespread use of this drug for both hospitalized and unhospitalized patients since its introduction approximately three years ago, only 2.3 per cent of the strains of *M. tuberculosis* isolated from untreated patients showed significant primary resistance to isoniazid *in vitro*.

None of the patients in this series had tubercle bacilli significantly resistant to both streptomycin and isoniazid, as was recently observed in a case reported by Katz, Storey, and McCormick (5).

It can be concluded, therefore, that, while strains of tubercle bacilli resistant to streptomycin and isoniazid can be demonstrated in the sputum of a small number of chest clinic patients with newly discovered and untreated active pulmonary tuberculosis, this does not, at present, represent a serious public health problem. To what extent primary drug resistance will become a problem in the future can be decided only by a continuous and more intensive type of study to include recently admitted hospital patients as well as clinic patients with newly discovered and as yet untreated active tuberculosis. Such an investigation is currently being conducted by the present writers in cooperation with the United States Public Health Service and will be the subject of future reports.

SUMMARY

Between July, 1953, and January, 1955, tubercle bacilli were isolated from 385 chest clinic patients with newly discovered and untreated active pulmonary tuberculosis. The patient-strains of tubercle bacilli were tested for susceptibility to streptomycin and isoniazid by the direct method. In 342 patients (89 per cent), the strains isolated were completely susceptible to both streptomycin and isoniazid. Of the 43 patients (11 per cent) from whom strains were isolated which showed any degree of resistance to either drug, only 6 (1.6 per cent of the total) had strains significantly resistant to streptomycin and only 9 (2.3 per cent of the total) had strains significantly resistant to isoniazid. None of the patients had organisms which were significantly resistant to both drugs.

SUMARIO

La Frecuencia de Cepas Estreptomicino- e Isoniacido-Resistentes del M. Tuberculosis en Enfermos con Tuberculosis Pulmonar Activa Recién Descubierta y No Tratada

Entre julio de 1953 y enero de 1955, se aislaron bacilos tuberculosos de 385 enfermos de una clínica torácica que tenían tuberculosis pulmonar activa recién descubierta y no tratada. Esos microbios fueron comprobados por el método directo en cuanto a susceptibilidad a la estreptomicina y la isoniacida. En 342 enfermos (89 por ciento), las cepas aisladas eran totalmente susceptibles tanto a la estreptomicina como a la isoniacida. De los 43 enfermos (11 por ciento) de quienes se aislaron cepas que mostraban algún grado de resistencia a una u otra droga, sólo 6 (1.6 por ciento del total) tenían cepas apreciablemente resistentes a la estreptomicina y sólo 9 (2.3 por ciento del total) tenían cepas apreciablemente resistentes a la isoniacida, según se define en este estudio. Ninguno de los enfermos tenía microorganismos que fueran apreciablemente resistentes a ambas drogas.

RESUME

Prédominance de souches de M. tuberculosis streptomycine- et isoniazide-résistantes chez des malades atteints de tuberculose pulmonaire active récemment découverte et non traitée

Entre juillet 1953 et janvier 1955, des bacilles tuberculeux ont été isolés chez 385 malades de dispensaire des voies respiratoires, qui étaient atteints de tuberculose pulmonaire en activité récemment découverte et non traitée. La sensibilité de ces microorganismes à la streptomycine et à l'isoniazide a été testée par la méthode directe. Chez 342 patients (89%), les souches isolées présentaient une sensibilité complète pour la streptomycine et l'isoniazide. Parmi les 43 patients (11%) chez lesquels on avait isolé des souches montrant différents degrés de résistance à l'un ou à l'autre de ces composés, chez 6 seulement (soit 1,6% de la totalité) des souches présentant une résistance significative à la streptomycine et 9 seulement (soit 2,3% de la totalité) des souches présentant une résistance significative à l'isoniazide, selon les normes de l'étude, ont été isolées. La présence de bacilles présentant une résistance significative aux deux composés n'a été observée chez aucun des malades.

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INFECTION WITH DRUG-RESISTANT TUBERCLE BACILLI[1]

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INTRODUCTION

Soon after streptomycin was first used in the treatment of tuberculosis, it became evident that strains of tubercle bacilli resistant to this drug emerged rapidly, especially in patients with cavitary disease. Because it was feared that the spread of streptomycin-resistant tubercle bacilli might become a serious public health problem, a study was begun in 1948 of the drug susceptibility of bacilli recovered from patients with newly diagnosed tuberculosis. As para-aminosalicylic acid (PAS) and isoniazid became available, the susceptibility of the patients' bacilli to these chemotherapeutic agents was likewise determined. In this way, it has been possible to determine the incidence of infection with drug-resistant tubercle bacilli and, in addition, the data obtained have led to a better understanding of the clinical course of individual infections and have served as a guide to therapy.

The first published reports of isolated cases of infection with streptomycin-resistant tubercle bacilli were made separately (1, 2); both patients were hospitalized employees whose tuberculosis was diagnosed in 1947. Reports of series of cases of infection with drug-resistant bacilli followed subsequently (3, 4), and an excellent summary of the literature on the subject has appeared recently in the *New England Journal of Medicine* (5).

Since 1948, approximately 600 patients with newly diagnosed tuberculosis have been admitted to this hospital and it was possible to culture tubercle bacilli from approximately 80 per cent of the group. Drug-susceptibility studies revealed that 10 (approximately 2 per cent) of these patients had been infected with drug-resistant tubercle bacilli. These cases are presented, with their management and course in the hospital, because of certain unusual features.

CASE REPORTS

Case 1. P. F., a 21-year-old male, became a tuberculosis hospital employee in August, 1949, at which time his cutaneous tuberculin reaction was negative. In May, 1950, he had an upper respiratory infection, and culture of the sputum at that time revealed tubercle bacilli. Two subsequent sputum examinations also yielded cultures of tubercle bacilli. Drug susceptibility studies revealed that the organisms were resistant[2] to 100 γ of streptomycin per ml. and susceptible to PAS. The tuberculin test was repeated and was positive. Roentgenograms of the chest at this time and on subsequent examinations failed to reveal a definite lesion in the lungs.

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

[2] Strains of tubercle bacilli were considered to be drug resistant when they grew as well in the presence of 10 or 100 γ of streptomycin or PAS as they did in the control tubes without drug. The greatest concentration used in the respective cases was either 10 or 100 γ.

The patient was kept under medical supervision for a year with treatment limited to moderate restriction of physical activity after working hours. He has remained well. This hospital employee was undoubtedly infected with streptomycin-resistant tubercle bacilli during the course of employment.

Case 2. F. N., an 18-year-old male, had a history of contact with a sister who received streptomycin therapy in a hospital in New York City. Chest roentgenograms of July and November, 1948, were negative; but a film of January, 1950, revealed pulmonary disease and the sputum contained tubercle bacilli. He was admitted to the hospital in March, 1950. Study of the admission sputum specimens revealed that his bacilli were resistant to 100 γ of streptomycin per ml. and susceptible to PAS. He was placed on dihydrostreptomycin and PAS daily from April to August, 1950, and on PAS alone from November, 1950, until discharge from the hospital for social reasons in March, 1951.

The clinical course was marked by slow and gradual improvement. The bacillary content of the sputum gradually diminished so that the final specimen was negative for acid-fast bacilli on microscopic examination. Roentgenograms of the chest revealed no particular clearing. Subsequently, the patient was hospitalized elsewhere on May 22, 1951, and received streptomycin and PAS until April, 1952, when the medication was changed to isoniazid, which was continued to February, 1953. A roentgenogram of January, 1953, revealed that there was no longer any evidence of cavity and the sputum had been negative for tubercle bacilli by all methods since February, 1952. As of January, 1954, he was considered to have minimal arrested disease and to be able to work six hours daily.

It is believed that the history of contact with a family member treated with streptomycin was of significance in this case and that the patient developed pulmonary tuberculosis caused by streptomycin-resistant tubercle bacilli.

Case 3. M. E. G., a 22-year-old female, had a history of contact with a husband who had died of tuberculosis in September, 1949, after treatment with streptomycin. A chest roentgenogram of July, 1948, was normal but, during the following months, the patient developed a productive cough, and films taken in November, 1948, revealed evidence of pulmonary disease. The sputum was positive for acid-fast bacilli. She was admitted to this hospital in February, 1949. Thoracoplasty was performed in May, 1950. The sputum continued positive for tubercle bacilli after the thoracoplasty and, because chemotherapy was contemplated, drug-susceptibility studies were carried out in February, 1951. The bacilli were found to be resistant to 10 γ of streptomycin per ml. and susceptible to PAS. She was treated with 1 gm. of streptomycin and 12 gm. of PAS daily for a total of one year. Tubercle bacilli disappeared from the sputum three months after the start of chemotherapy. A segmental resection of the left upper lobe was performed in October, 1951. Bacteriology of the resected material yielded tubercle bacilli resistant to 100 γ of streptomycin per ml. and susceptible to PAS. The patient was discharged from the hospital in February, 1952, and has been followed regularly in the outpatient clinic.

It is believed that this case represents an instance of a tuberculosis infection presumably acquired from the husband.

Case 4. H. H. L., a 31-year-old female, stated that she had a positive tuberculin test in 1940 while a student nurse. Her only known contact with tuberculosis was occupational, during work in tuberculosis hospitals. A chest roentgenogram of September, 1950, was normal but another film in September, 1951, revealed evidence of pulmonary disease, and she was referred to this hospital for admission in October, 1951. Only one culture positive for tubercle bacilli, in March, 1952, resulted from intensive sputum examinations. The bacilli were resistant to 10 γ of streptomycin per ml. and susceptible to PAS. She left the hospital in

June, 1952, to stay at home under the care of her private physician. She did well until September, 1954, when there was roentgenographic evidence of an exacerbation of her disease. She was then treated with PAS and isoniazid and, to date, it appears that healing of the disease has been excellent.

The evidence suggests that this patient was infected with drug-resistant tubercle bacilli with resulting pulmonary tuberculosis as a consequence of exposure in the course of her employment.

Case 5. H. L., a 43-year-old male, had positive skin reactions to both tuberculin and histoplasmin at the start of employment in a tuberculosis hospital in May, 1951. A roentgenogram of the chest showed a small, calcified nodule at the base of the right lung. This might have been due to either tuberculosis or histoplasmosis. A film taken in September, 1952, showed new infiltration in the right apex, and subsequent planigrams revealed evidence of cavitation in this area. The sputum was positive for tubercle bacilli, and drug- susceptibility studies revealed resistance to 10 γ of streptomycin and PAS per ml. and susceptibility to isoniazid. The patient was treated at home under the care of a private physician on a regimen of rest and streptomycin two to three times per week and 300 mg. of isoniazid daily. Although it was suspected that he was not too conscientious in taking his medication, there was roentgenographic evidence of slow but gradual improvement, and the sputum became negative for tubercle bacilli in January, 1953, and continued so. In the summer of 1954, the patient developed evidence of cirrhosis of the liver and died of this condition in October, 1954.

The evidence suggests that this patient was infected during employment in a tuberculosis hospital with tubercle bacilli which were resistant to both streptomycin and PAS.

Case 6. The patient, P. M., was a male medical student, aged 21, whose first tuberculin test early in 1952 was positive. During that summer he worked in the Pathology Department, assisting at autopsies. In September, 1952, a chest film revealed presumably tuberculous disease at the left apex, and culture of the gastric contents was positive for tubercle bacilli. The patient was admitted to this hospital in October, 1952, and, during the first two months of hospitalization, 4 sputum specimens yielded tubercle bacilli on culture. Drug-susceptibility tests revealed that the bacilli were resistant to 10 γ of streptomycin per ml., but susceptible to PAS and isoniazid. Chemotherapy was started in January, 1953; the regimen was 1 gm. of streptomycin three times weekly, 12 gm. of PAS daily, and 125 mg. of isoniazid three times daily. The sputum "converted" after one month and has continued to be negative for tubercle bacilli since that time. In July, 1953, the apical posterior segment of the left upper lobe and two nodules in the adjoining lung were removed. Bacteriologic study of the resected material revealed tubercle bacilli by microscopy, but cultures were sterile. Chemotherapy was continued until February, 1954, and the patient was discharged four months later. He has remained well and has returned to his studies.

It is believed that this case represents the acquisition of a drug-resistant tuberculous infection while the patient was assisting at post-mortem examinations.

Case 7. J. D., a 46-year-old male, contracted an upper respiratory infection in February, 1953. A roentgenogram taken after ten days because of failure to improve showed evidence of bilateral miliary tuberculosis. He was admitted to this hospital in March, 1953, acutely ill and confused. Other significant abnormalities were limited to some tubercles in the left retina and a small nodule in the prostate. Pretreatment sputum specimens were positive for tubercle bacilli, and drug-susceptibility studies revealed resistance to 10 γ of streptomycin per ml. and susceptibility to PAS and isoniazid. Tubercle bacilli recovered from the

urine and blood at this time were resistant in the same degree to streptomycin. The patient was put on a daily regimen of streptomycin, 2 gm.; PAS, 12 gm.; and isoniazid, 600 mg., and there was marked clinical improvement after four days. Tubercle bacilli were not recovered from the sputum or urine after April, 1953. Streptomycin was reduced to 1 gm. daily in May, 1953, and PAS had to be discontinued in March, 1954, because of a skin rash. The patient left the hospital against medical advice in July, 1954, and discontinued drug therapy. At the time of discharge, the chest roentgenograms could be interpreted as showing no pulmonary disease. He has continued well and is able to work.

The source of tuberculous infection in this case is unknown. There is no history of family or other exposure to tuberculosis. His home is in a rural community in northern New York.

Case 8. G. P., a 29-year-old female, gave a history of recent dietary treatment for obesity. The weight dropped from 189 pounds to 106 pounds. In June, 1953, she noted the onset of fatigue, productive cough, sweats, and fever. Roentgenograms of the chest taken as part of a complete physical evaluation revealed evidence of pulmonary disease, and the sputum was positive for tubercle bacilli. There was no known contact with tuberculosis. She was admitted to the hospital in July, 1953. Sputum specimens obtained at this time contained tubercle bacilli, and she was treated promptly with a combination of dihydrostreptomycin, 1 gm. three times weekly, and 250 mg. of isoniazid daily. Shortly thereafter the pretreatment drug-susceptibility tests revealed that the bacilli were resistant to 10 γ of streptomycin per ml. As a consequence, 12 gm. of PAS were added to the daily medication. All sputum examinations have been negative since one month after chemotherapy was begun. Her clinical course has been excellent. The patient remains in the hospital, and roentgenograms of the chest show marked resolution of the disease.

In this case the source of infection with resistant bacilli is unknown. As in the previous case, the patient's home was in rural New York.

Case 9. D. R., a 22-year-old male, was admitted to the hospital in October, 1954. Although he admits no known contact with tuberculosis, he worked on the psychiatric ward in a New York City hospital from October, 1952, to March, 1953. In the summer of 1954, he developed malaise, and a roentgenogram of the chest, obtained in September, showed evidence of active tuberculosis. He was referred to this hospital in October. The sputum contained tubercle bacilli which were found to be resistant to 10 γ of both streptomycin and PAS per ml., but susceptible to isoniazid. Prior to the final reading of the drug-susceptibility tests, the patient was placed on streptomycin, 1 gm. three times weekly; PAS, 12 gm. daily; and isoniazid, 300 mg. daily. In view of the drug resistance and because he was not tolerating PAS well, medication was changed to viomycin and isoniazid. He continues acutely ill and the course to date has not been favorable. In January, 1955, it was found that the patient's bacilli had become resistant to 10 γ of isoniazid per ml.

Although this patient denies any known contact with tuberculosis, his residence in New York City and his work in a hospital would seem to have afforded ample opportunity for acquiring infection with drug-resistant tubercle bacilli.

Case 10. L. G., a 28-year-old male, had a chest survey film in June, 1948, which showed bilateral upper lobe disease. A roentgenogram of the chest in the outpatient clinic in September, 1948, revealed similar abnormalities. There was no known contact with tuberculosis, but the history revealed an illness in 1945 which had been diagnosed "pneumonia." The patient was admitted to the hospital for study.

The patient's cutaneous reaction to 10 mg. of Old Tuberculin (OT) was negative. Repeated sputum examinations and bronchoscopic specimens were all negative for tubercle

bacilli and fungi. Examination of a lymph node biopsy specimen showed chronic nonspecific infection. The patient gained weight rapidly after admission to the hospital. In December, 1948, acid-fast bacilli were found in the sputum and the tuberculin reaction became positive. A chest roentgenogram at this time showed a slight increase in pulmonary infiltration. The sputum continued intermittently positive for acid-fast bacilli and, in September, 1949, a left anterior chest wall abscess developed and the drainage contained tubercle bacilli. Chemotherapy was instituted in October, 1949; the patient received 1.5 gm. of streptomycin and 12 gm. of PAS daily for three months and, after an interval of six weeks, PAS alone for almost eight months.

Pretreatment studies of tubercle bacilli recovered from the sputum in September, 1949, revealed that the organisms were resistant to 100 γ of streptomycin per ml. and susceptible to PAS. Tubercle bacilli subsequently isolated from the sputum, urine, chest wall drainage, and pus from the right epididymis showed the same degree of resistance to streptomycin. The patient did well and was discharged from the hospital in December, 1950. He was readmitted in June, 1952, because of a left epididymitis. The sputum was negative for tubercle bacilli but organisms isolated from the urine were streptomycin-resistant and PAS-susceptible. In July, 1952, he developed symptoms of meningitis which was thought to be tuberculous although tubercle bacilli were not found in the cerebrospinal fluid. Treatment with streptomycin, PAS, and isoniazid was begun immediately; within the week, however, *Cryptococcus neoformans* were identified in the cerebrospinal fluid and sulfonamides were added to the medication. These organisms were isolated twice from the cerebrospinal fluid and from the sputum on several occasions during this period. The patient showed gradual improvement. The left epididymal sinus healed slowly, and after two months all urine cultures were negative for tubercle bacilli. The patient was discharged from the hospital in August, 1953. He has continued well and is able to do light work.

In summarizing this case, it is conceivable that the pulmonary lesion for which the patient was hospitalized in 1948 was due to cryptococcosis and that he later acquired infection with streptomycin-resistant tubercle bacilli.

DISCUSSION

Since 1948, infection with drug-resistant tubercle bacilli has been observed in a very small proportion (approximately 2 per cent) of the patients from whom organisms could be recovered for study on admission to the hospital with newly diagnosed tuberculosis. The histories in 8 of the 10 cases revealed ample opportunity for infection with resistant bacilli; in only 2 cases was the source of infection unknown. Six of the infections were undoubtedly acquired in hospitals, four of these in tuberculosis hospitals. The tubercle bacilli were primarily resistant to streptomycin in 8 cases and to both streptomycin and PAS in the remaining 2 cases.

Thomas and associates (3) reported a group of nine cases; in four, the bacilli were resistant to streptomycin; in another four, to PAS; and, in one, the bacilli were resistant to both of these drugs. In an addendum to their report, the writers recorded six additional cases of primary infection with PAS-resistant tubercle bacilli. Thus far, the present writer has seen no case of infection with bacilli resistant to PAS alone.

Cummings and Livings (4) carried out streptomycin-susceptibility tests on the cultures of 1,166 patients admitted to Veterans Administration hospitals who had no history of previous drug therapy for tuberculosis. Thirty patients (2.6

per cent) were found to harbor tubercle bacilli resistant to 10 γ of streptomycin per ml.; a history of contact with tuberculosis was given by seventeen of these. In nineteen of twenty-six patients who received chemotherapy, the disease responded favorably to treatment with a combination of streptomycin and either PAS or isoniazid. One additional patient's disease responded well to streptomycin alone.

Nine of the patients in the present report were treated with a combination of streptomycin and PAS or isoniazid, or with all three drugs, and all except one had a satisfactory clinical and roentgenographic course. Two patients had resectional surgery. A good result from chemotherapy and hospital care was obtained even in miliary tuberculosis (*Case 7*) and in a patient with extrapulmonary tuberculosis and cryptococcus meningitis (*Case 10*). It is not surprising that the patient whose course was unsatisfactory represented one of the two cases infected with bacilli resistant to both streptomycin and PAS. This is also the only case in the series in which the tubercle bacilli became resistant to another drug (isoniazid) after chemotherapy.

The ultimate outcome of these cases is not yet known; nor can one be certain that the patient's disease benefited from the administration of the drug to which the bacilli showed resistance. Nevertheless, some benefit might be expected if the patient harbored a mixture of susceptible and resistant organisms. It would seem most likely that the good results were mainly due to the drugs to which the bacilli were susceptible and to the period of hospital care, although the first case in this group did well without chemotherapy, as did the case reported by Furtos and Doane (1).

In the light of present knowledge, it is concluded that the incidence of infection with drug-resistant tubercle bacilli will become even more infrequent in the future as a result of multiple-drug therapy and surgical procedures, both of which tend to hinder the emergence of drug-resistant strains of tubercle bacilli. In spite of the low frequency of infection with drug-resistant organisms in newly diagnosed cases of tuberculosis, it seems wise to continue such drug testing in order to identify such an infection promptly. Furthermore, the availability of several chemotherapeutic agents makes effective treatment of these patients less difficult than was anticipated when only one or two antituberculous drugs were available.

SUMMARY

Ten cases of pulmonary infection with drug-resistant tubercle bacilli are reported following a five-year study of 600 cases of newly diagnosed tuberculosis. The presumptive source of contact could be established in all but 2 of these. The strains of tubercle bacilli were primarily resistant to streptomycin in 8 patients and to streptomycin and para-aminosalicylic acid (PAS) in 2 patients. One patient did well without chemotherapy and 8 made satisfactory recoveries after multiple-drug therapy which included streptomycin. Only the tenth (and most recent) patient has an unfavorable outlook. It is concluded that, while infection with drug-resistant tubercle bacilli occurs in a small percentage of cases, it is not likely to become a serious public health problem. It is further concluded that the

majority of the patients infected with bacilli resistant to an antituberculous drug can, nevertheless, be treated successfully.

SUMARIO

Infección por Bacilos Tuberculosos Fármacorresistentes

Descríbense 10 casos de infección pulmonar por bacilos tuberculosos fármacorresistentes, observados en un estudio de 600 casos de tuberculosis recién diagnosticada en un período de cinco años. En todos los casos, menos 2, pudo establecerse el foco presuntivo de contacto. Las cepas de bacilos tuberculosos eran primariamente resistentes a la estreptomicina en 8 enfermos y a la estreptomicina y al ácido para-aminosalicílico (PAS) en 2. Un enfermo lo pasó bien sin quimioterapia y 8 sanaron satisfactoriamente despues de una polifármacoterapia que comprendió estreptomicina. Unicamente el décimo (y más reciente) enfermo tiene un pronóstico desfavorable. Dedúcese que, mientras la infección por bacilos tuberculosos fármacorresistentes no afecte más que a un pequeño porcentaje de los casos, no es susceptible de convertirse en problema sanitario de gravedad. Dedúcese además que a la mayoría de los sujetos infectados por bacilos resistentes a una droga antituberculosa, puede, sin embargo, tratárseles con éxito.

RESUME

Infection par bacilles tuberculeux résistants aux antibactériens

Rapport relatif à dix cas d'infection du poumon par des bacilles de Koch résistants aux agents antituberculeux au cours d'une étude de cinq années d'observation de 600 cas de tuberculose diagnostiquée précocement. La source présumée de la contamination a été établie dans tous les cas sauf 2. Les souches de bacilles tuberculeux initialement résistantes à la streptomycine ont été découvertes chez 8 patients, et à la streptomycine et à l'acide para-aminosalicylique (PAS) chez 2 patients. L'amélioration a été obtenu sans chimiothérapie chez un patient et 8 patients ont présenté des guérisons satisfaisantes après un traitement antibactérien combiné comprenant la streptomycine. Seul le dixième cas (le plus récent) présente un pronostic défavorable. En conclusion, l'existence d'un faible pourcentage de cas d'infection par des bacilles résistants aux antibactériens ne paraît pas devoir constituer un sérieux problème d'hygiène publique. L'auteur conclut également que la majorité des malades infectés par des bacilles résistants aux agents antituberculeux peuvent être néanmoins traités avec succès.

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THE CHARACTER OF TUBERCULOUS CAVITIES AS SEEN IN SURGICALLY RESECTED SPECIMENS[1]

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INTRODUCTION

According to Laennec, when an area of tuberculous matter undergoes liquefaction and establishes communication with a bronchus so that the contents can be emptied out, a cavity is formed. There has been a tendency to think of the tuberculous cavity as being an essentially stereotyped pathologic lesion, especially as seen at autopsy. If a large number of surgically extirpated lungs are reviewed, however, it soon becomes evident that many gross and microscopic variations of the tuberculous cavity warrant careful study and description.

Auerbach and his co-workers (1–3) were among the first to describe anatomic changes in the tuberculous lesion attributable to the administration of streptomycin. Undoubtedly, other agents that are used today in addition to, or instead of, streptomycin have a similar effect; nevertheless, there is some reason to believe that isoniazid may be largely responsible for the unusual type of cavity healing termed "open healing." A considerable variation in the pathologic lesions seen in surgically resected lungs is to be expected because the disease process is interrupted at different stages of healing, whereas autopsy specimens usually present the picture of terminal disease.

OBSERVATIONS

The present study consists of a review of 335 consecutive cases of pulmonary tuberculosis studied between January 1, 1950, and July 1, 1954. In 240 cases of this group, cavitation was evident in the resected specimens. In figure 1, the ratio between cavitary and noncavitary lesions for each year during the five and one-half years is graphically presented.

Method

The surgically resected segments, lobes and lungs, were fixed soon after removal by filling the bronchial tree with Kaiserling I solution under slight pressure so as to distend the lungs to a size comparable to their natural inflated state. This method of handling the specimens has considerable merit because it rapidly and uniformly fixes the tissue, preserves the configuration of cavities, and makes possible maximum color restoration if the Kaiserling method is carried to completion. Some time after treatment with Kaiserling I (twelve hours or longer), the lungs were sectioned into thin slices (0.5 to 1.0 cm. in thickness) and the pathologic changes were correlated with the preoperative roentgenograms. Representative sections were taken and stained with hematoxylin and eosin. In the cases of "open healing," many sections were stained with Masson trichrome and Ziehl-Neelsen stains.

An attempt was made to classify the different types of cavitary lesions, taking into

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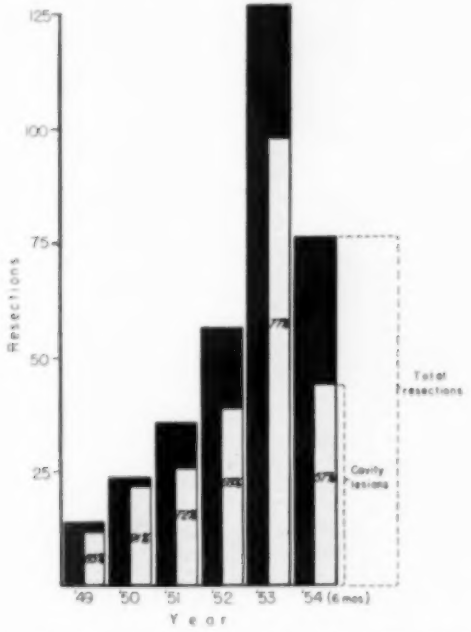

FIG. 1. The relationship between the number of patients with cavitary and noncavitary lesions over a five and one-half year period. Note that the percentage of patients with cavities is less today than at the beginning of the study.

TABLE 1

CLASSIFICATION OF THE CAVITIES FOUND IN 240 SURGICALLY RESECTED SPECIMENS

Type	Character of Cavity	Pneumonectomy	Lobectomy	Segmentectomy	Total	Per Cent
I	Thin-walled cavity (with a minimal amount of fibrosis about it)	5	16	5	26	10.8
II	Thick-walled cavity (fibrous wall and necrotic lining)	48	74	37	159	66.3
III	Cavity showing "open healing"	10	7	6	23	9.6
IV	Extensive disease with ulceration or cavitation	12	7	6	25	10.4
V	"Shelling out" of a solid lesion (tuberculoma)	1	4	2	7	2.9
	Total	76	108	56	240	100.0

consideration the configuration of the cavity, nature of the lining, thickness of the wall, and pericavitary parenchymal involvement. Five types of cavitary lesions as seen in the 240 cases are shown in table 1. They were further subdivided according to pneumonectomies, lobectomies, and segmentectomies.

TABLE 2

CHARACTERISTICS OF THE VARIOUS TYPES OF CAVITIES

Type	Number of Cavities	Location	Configuration	Lining Membrane	Wall	Adjacent Parenchyma	Communicating Bronchi
I	Single	Apical	Spherical	Necrotic; shaggy	Thin; very little fibrosis	Consolidated	Some endobronchitis
II	Single	Apical	Spherical or irregular	Granular to shaggy	Thick; marked fibrosis	Compressed; fibrosis	Some endobronchitis to bronchiectasis
III	Single or multiple	Usually apical	Spherical	Smooth; glistening	Thin; fibrous	Uninvolved	No significant change
IV	Multiple	Extensive involvement	Irregular	Necrotic; shaggy	Variable	Pneumonic involvement	Some endobronchitis to bronchiectasis
V	Usually single	Upper lung field	Irregular	Granular	Thin; fibrous	Uninvolved	Little change

In 45 cases, material was aspirated from the bronchial tree prior to fixation and this aspirate was examined by stained smear, culture, and/or animal innoculation (table 2). Inasmuch as the primary interest was in the anatomic and morphologic changes, it was considered impractical to mutilate the lung in order to carry out bacteriologic studies directly from the lesions.

The classification of the various types of cavitary lesions was based on their gross characteristics, and the principal features of each type are listed in table 2. The microscopic findings, roentgenographic interpretations, antimicrobial, and adjunctive collapse therapy of each group are given below:

Thin-Walled Cavities

Twenty-six (10.8 per cent) lesions showed thin-walled cavities, and the type of cavity seen is shown in figure 2*b*. There was a necrotic lining with little or no fibrous reaction apparent in the wall, and the parenchyma adjacent to it was consolidated due to atelectasis or pneumonitis. Microscopically, the cavity was seen to be lined by some acute inflammatory cells and necrotic debris. Beneath this a disorganized layer of fibroblasts, chronic inflammatory cells, an occasional giant cell, and a few blood vessels were present. The adjacent lung parenchyma was the seat of an organizing pneumonia and atelectasis. A review of the roentgenograms showed a persistent thin-walled cavity which became apparent a few months prior to surgery and increased in diameter at a rapid rate. The thin-walled cavity in the left upper lobe is illustrated in figure 2*a*. A right pneumothorax was maintained from May, 1946, to January, 1948. The patient underwent a right thoracoplasty in May, 1950, and pneumoperitoneum was initiated in July, 1951, and abandoned just prior to the resective surgery. The patient received dihydrostreptomycin and PAS from August 4 to October 22, 1948, and again from May 23 to July 25, 1950, for a total of four and one-half months. PAS alone was administered from March 12 to October 28, 1951. A segmentectomy of the left upper lobe was performed on October 28, 1951.

Thick-Walled Cavities

As one would expect, the group of thick-walled cavities comprised the majority of the lesions seen. As shown in table 1, 169 (66.3 per cent) of the total

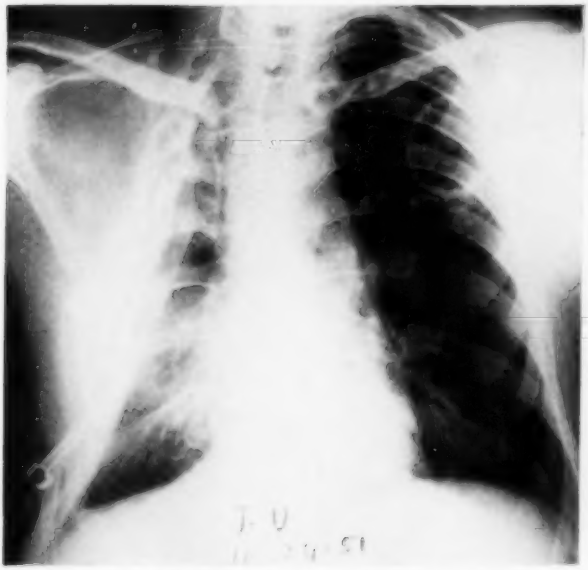

FIG. 2*a*. (J. V.) Thin-rimmed cavity wall noted in the periphery of the left upper lobe. Evidence of the right thoracoplasty is seen. This picture was taken one week prior to segmentectomy.

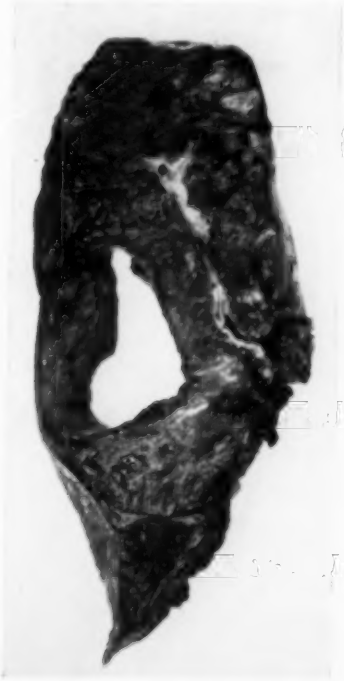

FIG. 2*b*. (J. V.) Thin-walled cavity in the apical-posterior segment of the left upper lobe. Note the absence of fibrous tissue in the wall and the narrow rim of consolidated parenchyma about the cavity.

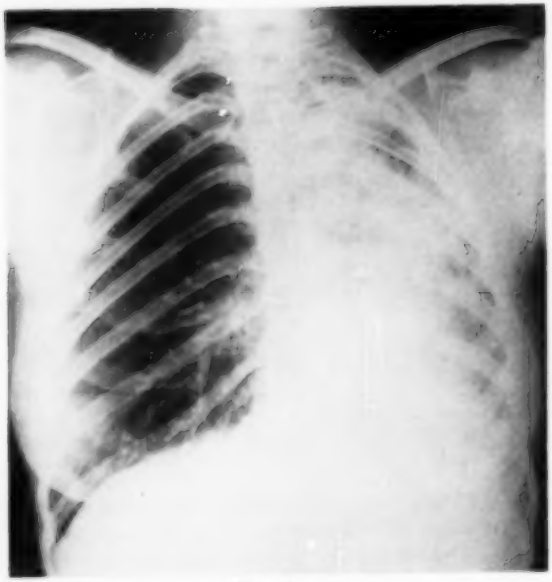

FIG. 3a

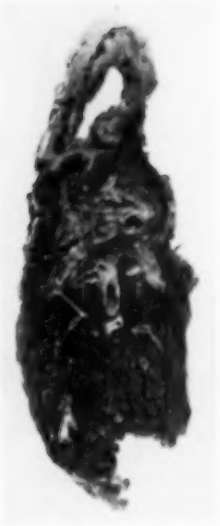

FIG. 3b

FIG. 3a. (L. J.) Roentgenogram taken two days prior to pneumonectomy. This patient did not receive any collapse therapy.

FIG. 3b. (L. J.) Left lung showing a chronic cavity in the apex with marked proliferation of fibrous tissue and a heavy pleural cap. There is a thin necrotic lining. A second cavity is seen off the hilus.

lesions showed cavitation of this sort. The surgeons elected to do a lobectomy for this type of lesion in approximately one-half of the patients (table 1). Figure 3*b* is a typical example of a chronic thick-walled apical cavity. Grossly, there was a necrotic lining coating a vascular layer of granulation. A heavy fibrous wall surrounded the cavity, with compression of the adjacent lung parenchyma, and there was marked pleural thickening. Microscopically, the cavity was lined by necrotic material with a few inflammatory cells. Beneath this lining was a fairly well-organized fibrous tissue layer which had many lymphocytes scattered throughout. There was a paucity of giant cells and, toward the periphery, the fibrous tissue was very compact and hyalinized. The bronchi in the vicinity showed marked bronchiectatic change.

Serial roentgenograms showed evidence of cavitation for eleven months prior to surgery; the film taken the day before surgery is illustrated (figure 3*a*). This patient received dihydrostreptomycin and PAS for ten and one-half months from April 27, 1950, until the left pneumonectomy was performed on March 14, 1951.

Cavities Showing "Open Healing"

Twenty-three of the lesions (9.6 per cent) showed the unusual and intriguing type of healing designated "open healing." Grossly, the lining membrane was

FIG. 4*a*. (L. F.) Huge cavity in the upper portion of the left chest. Admission film (February 27, 1953) showed evidence of cavitation. Pneumoperitoneum was instituted on August 12, 1953.

FIG. 4*b*. (L. F.) Upper portion of the left lung showing a huge thin-walled cavity with a smooth lining and several trabeculae coursing through it. A striking example of "open healing."

smooth and glistening and, in many instances, it was transparent (figure 4*b*). In 7 of the 23 lesions, small granulations and/or ulcerations were seen in some areas. These foci were considered "unhealed," as will be described later. By and large, the majority showed only a thin fibrous wall, and the adjacent lung showed no specific alteration. Representative microscopic sections from these cavities revealed a monotonous picture of a dense fibrous tissue wall with some hyalinization and a few scattered lymphocytes. Occasionally, an area of calcification was noted and single or aggregate atypical giant cells were seen. Some of these giant cells contained laminated, dark purple material not unlike Schaumann bodies seen in sarcoidosis. The areas of yellow granulation mentioned above were atypical tubercles, with variable degrees of necrosis, a few epithelioid cells, atypical giant cells, calcification, and fibrous tissue. The ulcerated areas represented granulation tissue or loosely arranged and disorganized young fibrous tissue. In both instances, there was always a fairly heavy wall of compact fibrous tissue beneath the lesions, and some of the yellow granulations were covered by a sparse layer of fibrous tissue.

Several blocks of tissue from each cavity were processed, and sections from different levels in each block were stained for acid-fast bacilli, but none were found. Practically all of these cavities had a demonstrable communication with one or more bronchi; however, the bronchi did not show any unusual change.

These lesions were differentiated from emphysematous bullae or bronchiectatic cavities on the basis of serial preoperative roentgenograms, lack of accessory bronchial structures, paucity of anthracotic pigment, anatomical location, et cetera. It should be pointed out that none of the cavities showed epithelization of the lining. In some instances, the bronchial epithelium extended for a short distance into the cavity.

A review of the preoperative roentgenograms (figure 4*a*) showed that a cavity had been present from the day of admission. The cavity gradually increased in size. The film was obtained six weeks prior to surgery. This patient received streptomycin and PAS for a little longer than one year (February 28, 1953, to March 15, 1954) prior to surgery and isoniazid for two months prior to surgery. Twenty of the 23 patients in this group received isoniazid, and the other 3 received only dihydrostreptomycin or streptomycin and PAS.

Extensive Disease with Cavitation or Ulceration

Of the 25 (10.4 per cent) specimens from the patients with extensive cavernous disease, 12 were obtained through pneumonectomies, which indicates the degree of involvement of the affected lung. Most of these cases were interpreted clinically as "destroyed" lungs. Grossly, the entire upper lobe was seen to be involved (figure 5*b*) in a fibrocaseous process with irregular areas of ulceration and cavitation. There was some extension of the process across the interlobar fissure. Microscopically, little, if any, lung parenchyma remained. It was largely replaced by caseation necrosis, with varying degrees of fibrous tissue proliferation. Irregular areas of ulceration were noted. Serial roentgenograms revealed a caseous pneumonic type of involvement of the left lung with multiple cavities

FIG. 5a

FIG. 5b

FIG. 5a. (A. W.) Marked involvement of left lung seen on this roentgenogram taken day before pneumonectomy. Pneumoperitoneum had been maintained for one year.

FIG. 5b. (A. W.) Section through left lung showing marked involvement with fibrocaseous disease and several irregular excavations.

as early as March 28, 1951 (one day after admission to the hospital). The roentgenogram of August 5, 1952, may be seen in figure 5*a*.

This patient received dihydrostreptomycin and PAS for a period of two years and five months (March 30, 1950, to August 6, 1952) prior to surgery. Because of the character of the lesions seen in this group, the chemotherapy of all of the patients who showed this type of lesion was reviewed and was found to be theoretically adequate. Nine of the 12 patients subjected to pneumonectomy had received pneumoperitoneum for some time preoperatively.

Cavities Formed by the "Shelling-Out" of a Tuberculoma

Seven (2.9 per cent) of the cavities were believed to be tuberculomata (encapsulated caseous foci) which had undergone liquefaction and had partially "shelled-out." Grossly, it appears that the manner in which this type of lesion excavates is unusual and unpredictable as well. In figure 6*b*, several solid lesions

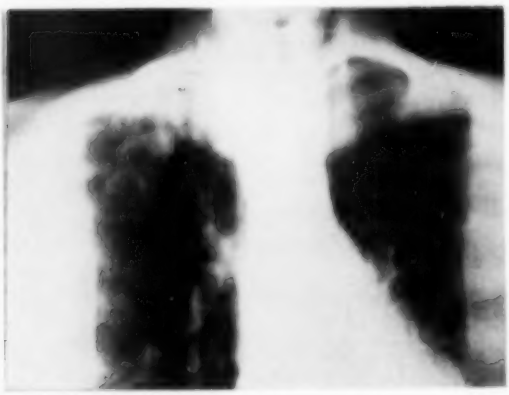

FIG. 6a. (A. C.) Tomogram showing densities in the right upper lobe with questionable cavitation.

FIG. 6b. (A. C.) Multiple solid lesions (tuberculomata) in the right upper lobe with "shelling-out" of one of these to form a small cavity.

may be seen, but one shows cavitation of one portion which appears to have begun peripherally. The tuberculoma is sharply demarcated from the surrounding tissue and has a thin translucent capsule about it. Invariably, single or multiple points are found about the capsule where the process is "out of bounds." The body of the tuberculoma usually has a laminated appearance and shows some anthracotic pigmentation.

The microscopic picture was that of central caseation with variable amounts of fibrous tissue encircling it. Almost without fail, in the "out of bounds" areas, there were small active tubercles, making the lesion a source of potential trouble. Serial roentgenograms are usually conclusive in establishing the nature of the lesion, but because of the density of the "tumor," a small excavation may not be seen on the films. In the case illustrated, postero-anterior, lordotic, and kyphotic films showed some contraction of right apical lesions. A cavity was suspected and laminagrams were made (figure 6*a*). The antimicrobial therapy in this case consisted of approximately nine months of streptomycin and PAS prior to surgery (May 26, 1953, to February 15, 1954), at which time the right upper lobe was removed.

DISCUSSION

The character of a tuberculous cavity is undoubtedly influenced by several factors. The configuration of the lesion may be altered considerably by artificial attempts at cavity closure, such as pneumothorax, thoracoplasty, pneumoperitoneum, et cetera. This was most noticeable in those lungs which were removed following thoracoplasty. Moreover, the administration of antimicrobial agents (streptomycin, para-aminosalicylic acid, and isoniazid) is known to enhance the healing processes. Since the early days of treatment with streptomycin, numerous reports have described the effect of this drug on the healing of tuberculosis. It has also been pointed out that the perifocal effusion about tuberculous lesions is of lesser degree. In reviewing the specimens in the present study, it was noted that there was considerable proliferation of fibrous tissue about the blood vessels and lymphatics and along the alveolar septa scattered throughout the tissues and not generally related to any specific lesion. This generalized fibrous tissue proliferation was usually accompanied by some degree of endarteritis.

Those cavities classified as early and showing little or no fibrous tissue response in their walls were thought possibly to represent instances in which inadequate antimicrobial therapy had been given. The fact that 38 per cent of the patients in this group received therapy for less than six months, 46 per cent received therapy for six to twelve months, and only 16 per cent received more than twelve months of therapy prior to surgery tends to support this belief. Undoubtedly, additional factors have been instrumental in thwarting the healing process as well.

One of the most interesting groups in the study included those lesions termed "open healing." Oddly enough, none of the specimens from the resections of 1949 and 1950 showed any instances of "open healing," and only one case in 1951 was recorded. In 1953, however, there were 13 such cases.

TABLE 3

SUMMATION OF THE BACTERIOLOGIC DATA ON THE MATERIAL ASPIRATED FROM THE BRONCHIAL TREE OF 45 CONSECUTIVE SURGICAL SPECIMENS

Type of Cavity	Number of Cases Studied	Negative	Positive	Positive Results Obtained by:					
				SM*	CU*	GP*	SM CU	SM GP	CU GP
Thin-walled	7	3	4	3	1	2	1	1	0
Thick-walled	26	15	11	7	7	7	5	3	4
"Open-healing"	4	4	0	0	0	0	0	0	0
Ulceration—widespread	6	4	2	0	1	2	0	0	1
"Shelling-out" tuberculoma	2	1	1	0	1	0	0	0	0
Totals	45	27	18	10	10	11	6	4	5

* SM—stained smear of sediment; CU—culture on artificial media; GP—guinea pig inoculation.

It would seem that this type of healing is related to present-day chemotherapy and more specifically to the administration of isoniazid. Johnson and Hewitt described the roentgenographic findings on twenty-two patients as being cyst-like cavities. Four of their patients were operated upon, and the specimens confirmed the roentgenographic interpretations.

In a recent publication, Pagel and Simmonds (6) gave detailed histologic observations on the effect of chemotherapy on the cavity wall. They were of the opinion that there is a cleansing of the cavity walls, with preservation of the cavity space. This occurs without epithelization of the cavity lining, even though there may be epithelium present in the bronchocavitary junction. This re-epithelization is considered to be an important factor in "open healing," according to Auerbach (7), who has observed an increased number of "open healed" cavities following the prolonged use of chemotherapy. Another factor which contributes to greater incidence of "open healing," he believes, is the late liquefaction of inspissated cavities.

In describing the modification of tuberculous lesions in patients treated with isoniazid, de Figueiredo and de Paola (8) point out that five patients showed complete healing of the cavity wall without any evidence of epithelization save for one cavity which previously had been drained.

Finally, the character of a tuberculous cavity may depend upon the nature of the pre-existing lesion, with special reference to solid lesions commonly termed tuberculomata. In the present series there were 7 cases of "shelling-out" of a tuberculoma, giving rise to an irregular cavity of variable dimension. Microscopic examination of a tuberculoma will show small foci of activity at the periphery which liken it to a smoldering fire. Portions of the lesions may undergo liquefaction necrosis, establish communication with an adjacent bronchus, and form a cavity. Widespread fibrocaseous disease within a lobe or a lung likewise may undergo excavation and form a bizarre pattern.

In table 3, the results on 45 consecutive cases show that positive bacteri-

ologic evidence of tuberculosis was obtained in 40 per cent of the cases. It should be pointed out that 4 cultures were contaminated, guinea pigs were not inoculated in 5 cases, and in 8 cases the results of microscopy were not reported.

SUMMARY

A review of the surgically resected specimens from 335 patients with pulmonary tuberculosis at the City of Chicago Municipal Tuberculosis Sanitarium revealed that 240 specimens showed cavitary lesions which could be classified into five different types: thin-walled cavitary, thick-walled cavitary, "open-healing," widespread fibrocaseous disease with ulceration, and "shelling-out" of solid lesions (tuberculomata or encapsulated caseous foci).

The most unusual lesion was that termed "open-healing," which was formerly considered a rare phenomenon but accounted for 9.6 per cent of all the cavitary lesions in this series.

SUMARIO

La Naturaleza de las Cavernas Tuberculosas Según se Observa en Ejemplares Resecados Quirúrgicamente

El repaso de los ejemplares resecados quirúrgicamente de 335 tuberculosos pulmonares en el Sanatorio Municipal para Tuberculosis de la Ciudad de Chicago reveló en 240 enfermos lesiones cavitarias que podían clasificarse en cinco distintas formas: cavitarias de paredes delgadas, cavitarias de paredes gruesas, de "cicatrización abierta", enfermedad fibrocaseosa difusa con ulceración, y "decapsulación" de lesiones macizas (tuberculomas o focos caseosos encapsulados). La lesión más extraña fué la denominada "cicatrización abierta", considerada anteriormente como fenómeno raro, pero a la que correspondió un 9.6 por ciento de todas las lesiones cavitarias de esta serie.

RESUME

Le caractère des cavernes tuberculeuses observées dans les pièces de résection

L'analyse de pièces provenant de résections pratiquées dans 335 cas de tuberculose pulmonaire au Sanatorium municipal de la tuberculose de la ville de Chicago, a révélé que 240 malades présentaient des lésions cavitaires qui pouvaient être classifiées selon cinq types différents: caverne à paroi mince, caverne à paroi épaisse, "open healing", lésions fibrocaséeuses étendues avec ulcération et "shelling-out" de lésions denses (tuberculome ou foyers enkystés). La lésion la plus surprenante était celle de type "open healing", *(lésion ouverte dans une bronche et en voie de cicatrisation)* qui était antériuerement considéré comme un phénomène rare, mais qui représentait 9,6% du total des lésions cavitaires de cette série.

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ALLERGY IN EXPERIMENTAL MOUSE TUBERCULOSIS[1]

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INTRODUCTION

The reaction of the mouse to experimental tuberculosis has been the subject of many controversial reports. Until recently, this animal appeared to occupy a peculiar position among those animal species naturally subject to tuberculosis or used for studying experimentally the pathogenesis of this disease. This position was related to the following differences, some of which have already been found to be apparent rather than actual:

A high natural resistance to artificial infection
The absence from the resulting lesions of several of the cellular components of the classical tubercle believed to be important in acquired resistance
An inability to acquire specific resistance to the disease
A failure to develop tuberculous allergy

There is evidence that the resistance of mice to infection with virulent bacilli depends mainly on the route of inoculation. If the bacilli lodge in the lung, lesions develop readily and rapidly in this organ and it has been shown (1, 2) that C57 black mice inoculated intranasally and kept on a deficient diet are as susceptible to infection as guinea pigs inoculated intramuscularly.

Swedberg (3) reviewed the literature on the development of specific resistance, and from this and his own work concluded that mice can acquire resistance. This view was confirmed by the recent work of Dubos, Pierce, and Schaefer (4).

The inference that acquired resistance in the mouse should be accompanied by allergic phenomena and the development of a specific cellular reaction led to the studies reported in the present paper. The susceptibility of guinea pigs to anaphylactic and allergic reactions, including tuberculin shock, is well recognized. Mice and rats, on the other hand, are reported to be relatively insensitive to the anaphylactic type of reaction, probably because of their histamine tolerance. Trethewie (5), comparing the LD_{50} of histamine given intravenously to guinea pigs, mice, and dogs, found that mice were eight hundred times as resistant as guinea pigs to the drug. Dougherty (6) found that, if mice sensitized to horse serum were adrenalectomized two hours before shock, they became two thousand times as susceptible to anaphylaxis as similarly sensitized, sham-operated mice. Their relative resistance could be fully restored by administering secretions of the adrenal cortex to the adrenalectomized mice.

The experiment recording the results of infecting mice with tuberculosis after

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adrenalectomy is included to support the view that the allergic response of mice bears no direct relationship to their histamine sensitivity or to their adrenocortical mechanism. Tuberculous allergy in particular is a cellular rather than a humoral phenomenon, not obviously related to histamine sensitivity.

The published reports of experiments to study the tuberculin sensitivity of mice are conflicting. Gerstl and Thomas (7) found that mice infected with human or bovine tubercle bacilli remained insensitive to skin tests with purified protein derivative (PPD). Moreover, the growth rate and physical appearance of tissue cultures from normal and tuberculous mice were unaffected by adding one thousand skin-test doses of PPD to the medium. Their conclusion that mice are naturally anergic to tuberculin has been accepted by several later workers in this field. Thus, Holden, Seegal, and Adams (8), detecting no specific reaction to tuberculin by fragments of splenic tissue cultured from mice one month after infection, concluded this to be due to the natural anergy of mice reported by Gerstl and Thomas.

Favour (9) found that lymphocytes, but not granulocytes, from the peripheral blood or spleen of tuberculous mice were lysed *in vitro* by tuberculin. Because both of these cell types from man and guinea pigs were lysed by tuberculin and since his attempts to obtain a positive skin reaction were unsuccessful, Favour suggested a possible relationship between the anergic state in the mouse and the resistance to lysis of granulocytes. Further, having failed to show that the relative skin anergy was reversed by adrenalectomy, Favour (10) concluded that mouse leukocytic responses, as well as dermal reactions to tuberculin, differ from those in man and guinea pigs.

In the last few years, however, the possibility of a limited development of allergy by tuberculous mice has been re-examined. Hart, Long, and Rees (11) comment briefly on an observation that, although skin sensitivity cannot be demonstrated, a systemic reaction may follow the intravenous injection of PPD in a much larger dose than is required to produce a similar reaction in infected guinea pigs. Continuing this observation, Kirchheimer and Malkiel (12) reported that mice sensitized by either H37Rv or BCG strains could be fatally shocked with intravenous PPD as early as ten days after infection. No control mice appear to have been used in these tests, however, and they are therefore difficult to interpret.

The present results are based on studies of C57 black mice inoculated with living or dead *Mycobacterium tuberculosis* H37Rv by several routes or with BCG intraperitoneally. Groups of guinea pigs infected intradermally or intranasally with H37Rv were tested in parallel to compare the allergic response of the two species. The fate of stainable and viable bacilli in the lungs of intranasally inoculated mice and guinea pigs was also studied by weekly examinations, using fluorescence microscopy (13) and surface plating methods (1), respectively. The results, together with those of a preliminary examination of the relationship of the cellular changes in the mouse lung to the bacterial population, are included here.

MATERIALS AND METHODS

Cultural procedures: Dried ampoules of *M. tuberculosis* H37Rv, freshly recovered from a tuberculous animal, were held as a source of uniformly virulent tubercle bacilli. Every three months a Löwenstein slope was seeded from an ampoule and was refrigerated when well grown. Weekly subcultures from this stock were used as a source of three successive ten day cultures in Tween[8]-albumin medium (14). This third subculture was arbitrarily selected as the starting point for all experiments because it gave uniform dispersal of bacilli in the minimal number of subcultures after isolation from the diseased animal.

Standardization of the inoculum was effected in the following manner:

> After a light centrifugation to remove any larger clumps of bacilli, the culture was adjusted with 0.1 per cent albumin-water to a reading of 97 on an Evelyn photoelectric colorimeter, using a 660 filter. After centrifugation at 3,500 r.p.m. for twenty minutes, the bacilli were resuspended in albumin water to the original volume. It was found that a 10^{-4} dilution contained from 200 to 1,000 viable units per ml. as determined by surface plating counts.

All suspensions were counted in this manner when prepared but, because the results were not available for several weeks, the actual dose in any experiment was a dilution of the standard suspension anticipated to contain approximately the desired number of bacilli.

Suspensions of BCG were made from dried cultures prepared for human use by the Commonwealth Serum Laboratories. The number of viable organisms present in the resuspended vaccine at the time of inoculation was determined as above.

The killed suspensions of H37Rv were prepared from cultures grown for ten days in Tween[8]-albumin medium, phenolized to a final concentration of 2 per cent, and incubated at 37°C. for five days with daily shaking. The cells were then centrifuged out and taken up in Tween[8]-albumin medium to two thirds of the original culture volume. Sterility was confirmed by observing 0.5 ml. of the suspension on Löwenstein medium for six weeks.

Animals: The mice employed were of the C57 black strain introduced from America in 1947 and since bred as a pure line. The guinea pigs were from a short-haired strain, also maintained with no outcrossing for many years. The susceptibility of these guinea pigs to tuberculosis is high (2), and no natural infection has ever been detected among them. Prior to inoculation all animals were kept on a full balanced diet (2), and any subsequent alteration is noted in the text.

Tuberculin sensitivity tests: Tests were performed with Koch's Old Tuberculin[1] (OT) prepared for human use. PPD[2] was found to produce a similar intensity of footpad or skin reaction, but has not been used in routine testing.

Skin tests in guinea pigs consisted of inoculating approximately 0.1 ml. of a 1:50 dilution of OT in saline into the shaved skin of the thigh, flank, or back with a half-inch, medium-beveled, 26-gauge hypodermic needle. The appearance of an erythematous swelling at twenty-four hours, persisting for at least forty-eight hours, with or without a central area of necrosis, was considered a positive result, provided control animals showed no lesion at forty-eight hours other than the slight damage caused by the needle. The results were recorded as on the day of inoculation, rather than on the day of reading the reaction.

After unsuccessful attempts to perform intradermal inoculations into the thin skin of mice in various parts of the body, it was found possible to inoculate without difficulty into the metatarsal pads. For this purpose it was convenient to hold the animal by the loose skin of the neck and back, with the tail immobilized by the little finger, the ventral surface of the animal presented to the inoculator, and the head pointing obliquely downward. After swabbing the foot with 70 per cent alcohol, the plantar surface was tensed by the thumb and forefinger grasping the toes, and the tip of a short-beveled, 26-gauge needle

[1] Commonwealth Serum Laboratories, Parkville, Victoria.

could then readily be introduced into the distal aspect on one of the pads. With slight pressure on the plunger, the sole of the foot immediately surrounding the pad was distended into a circumscribed swelling approximately 3 mm. in diameter. If the needle accidently punctured the dermis, a subcutaneous swelling was produced extending along the whole plantar surface to the tarsus. Weekly tests were performed on alternate hind feet.

Normal mice showed no reaction at forty-eight hours to dilutions of OT ranging from 1:10 to 1:50, but the undiluted product caused severe inflammation leading to gangrene of the toes. The dilution finally selected was 1:25. When PPD was used, it was made up to equivalent strength (750 units in 2 ml. of sterile distilled water). While inoculation at this strength caused only a very transient swelling which disappeared within forty-eight hours in normal controls, allergic animals showed a persistent reaction.

Tuberculin shock in both mice and guinea pigs was induced by inoculating 0.1 or 0.2 ml. of OT intraperitoneally. Deaths which occurred in twenty-four to forty-eight hours were considered to be due to allergy and not to anaphylaxis, provided the lesions showed an intense hemorrhagic infiltration at autopsy. These doses, even when given repeatedly for several weeks, did not produce any ill effects in uninfected controls other than a transient abdominal discomfort.

Lung counts: Both species of animals were killed with coal gas when necessary. The lungs were removed aseptically into sterile saline, and all lobes on the left of the midline were ground with sterile sand. They were then diluted and counted by the surface plating method (1). The rest of the lung was placed in formalin.

Sections: Tissues were fixed in 10 per cent formalin and sections were stained with auramine-rhodamine (13) and also, in the case of the mice, with hematoxylin-eosin. To allow for an observed tendency for the distribution of stainable bacilli to vary with the distance of the section from the center of the tuberculous focus, serial sections were cut right through selected lesions. Representative sections at all levels were then examined before an opinion was reached concerning the number of bacilli present. For uniformity, the plates presented relate to sections as close as practicable to the center of a tubercle. The data on the pathologic changes are taken from sections adjacent to those in figures 4–13.

RESULTS

Cutaneous Reactions to Tuberculin in Mice

In more than 300 mice regularly tested by the footpad method for periods ranging from four to thirty-four weeks, the pattern of the allergic response recurred regularly.

For the purpose of this and later experiments, a reaction arbitrarily designated as *positive* (figure 1) consisted of a painful, reddened foot swollen to approximately twice its normal thickness. The reaction was marked at twenty-four hours, persisted strongly up to forty-eight hours, and then slowly reverted to normal in the next two days. To describe swellings of smaller dimensions, the term *intermediate reaction* was adopted until it is known whether it is justifiable to recognize degrees of response. Intermediate reactions were usually noted for one or two weeks before full conversion occurred, or in mice recovering from sublethal tuberculin shock, or when allergy was waning.

In the present report, *combined reactors* consist of all animals showing footpad swelling. These include the strong positive reactors but, because the latter are at present believed to represent complete conversion, a separate record of positive reactors is also given. It should be noted that reactions were often more marked at twenty-four than at forty-eight hours, so that a positive reaction at twenty-

FIG. 1. Tuberculin reaction forty-eight hours after inoculating 1:25 dilution of Old Tuberculin into the footpad of a C57 black mouse.

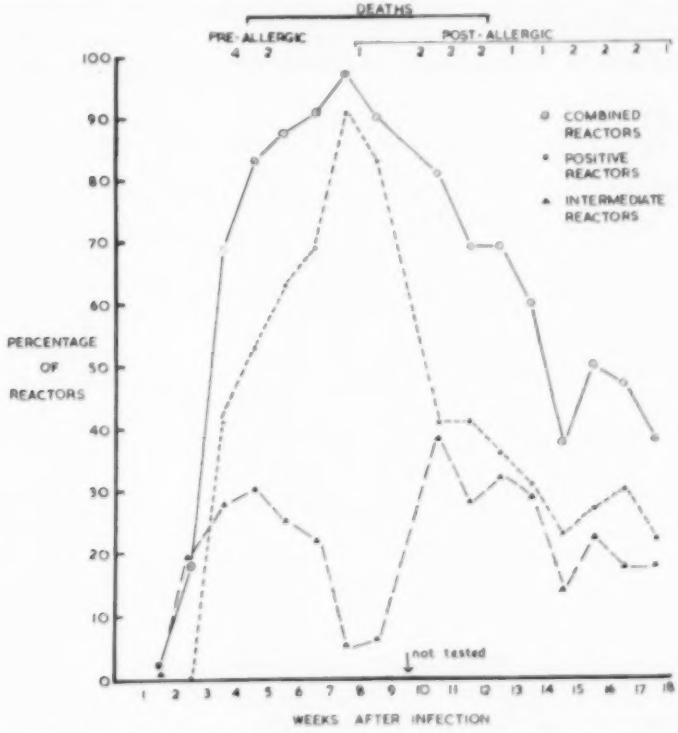

FIG. 2. Graph showing the progress of allergy in mice; 42 animals inoculated intranasally with 100 viable units were skin tested weekly from the tenth day with a dilution of 1:25 OT into footpad. Six of the original 42 died prior to conversion; 16 died after reversion to anergy; 30 control animals were consistently insensitive.

four hours might well be recorded as an intermediate at the final reading. The decision to read at forty-eight hours was taken because any nonspecific swellings in the controls had then subsided, while specific reactions still persisted.

The group of mice selected to illustrate the conversion pattern consisted of 42 animals inoculated intranasally with approximately 100 viable units of H37Rv and tested regularly for thirty-four weeks. These were adult male mice of which the precise age at inoculation is not known (*see* table 1), but half the number were three or four months old and the remainder more than six months old. These animals were still recognizable at the time of conversion because of the larger size of the older group, but later no such distinction was possible.

It has been observed in all of these studies that, in any intranasally infected group of mice, a few usually became ill and died between the second and sixth weeks after infection with virtually the whole of the lung tissue consolidated and the alveoli packed with bacilli (*see* figure 8). The majority, however, remained in good condition and survived for periods ranging from two months upwards. The proportion of animals which died in the early stages was partly but not entirely related to the size of the infecting dose, which was usually very small in the experiments under consideration (*see* table 3). From earlier experience of the effects of deficient diet on the susceptibility of mice to small infecting doses, it

TABLE 1

FOOTPAD TUBERCULIN REACTION OF INDIVIDUAL MICE IN RELATION TO CLINICAL CONDITION AND DEATH

Sixteen survivors after eighteen weeks from figure 2. Dose 100 viable units of H37Rv intranasally.

Mouse Number	Weeks Since Infection															
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	35	34
1	I*	D5														
2	−†	D1														
3	−†	D2														
4	−†	D2														
5	I*	−†	D3													
6	+	I	I*	−†	D1											
7	+	I	I	I	−	I*	−†	D4								
8	I	I	I	I	−	−	−†	−†	D1							
9	I	−	+	I	I	−	−*	−†	D6							
10	+	I	−	−	I	I	−*	−†	−†	D5						
11	I	−	−	I	I	I	I	−*	−†	D5						
12	+	I	I	+	−	I	I	+	I	+	+	+	+	+	+	+
13	+	I	I	I	I	I	I	I	+	I	+	+	+	+	+	I
14	I	I	+	+	I	I	−	−	I	I	I	+	+	+	I	I
15	I	−	I	I	−	I	−	I	I	I	−	−	I	I	+	I
16	I	−	+	−	−	I	I	I	+	I	+	+	+	+	+	I

Footpad reactions: + = positive; I = intermediate; − = negative; D1 to 6 = dead, respectively, 1, 2, 3, 4, 5, and 6 days after last test.

* Loss of condition.

† Emaciation.

seemed probable that a deficient diet might reduce the average survival time. Hence, the period of observation necessary to relate footpad sensitivity to survival might be shortened. The present group of mice was therefore placed on a deficient diet (2) from the time of infection.

In figure 2, the rate of footpad conversion and the subsequent allergic history of the group until the eighteenth week are shown. During this stage of the experiment, mice were not identified individually other than by an earmark when the foot reaction became negative. The appearance of fluctuations in the shape of the later stages of the reaction curves led to the segregation and individual study of the remaining mice (table 1).

It will be seen that the first positive reactions were recorded three and one-half weeks after infection, rather earlier than in other experiments (*see* table 3). It was also noted that all of the early reactions occurred in the old mice. This observation led to the experiment on mice of different ages recorded in table 2.

Six mice died without becoming allergic, but conversion in the others proceeded steadily so that, after seven and one-half weeks, 90 per cent of the surviving animals were showing a positive tuberculin reaction. The fact that at no time was the usual 100 per cent of positive reactions obtained (*see* table 3) is attributed to an unusually rapid progress of the disease in this group, leading

TABLE 2

THE INFLUENCE OF MOUSE AGE ON TUBERCULIN CONVERSION RATE
(Small Infecting Dose)

Age of Mice	4 Weeks			14 Weeks			24 Weeks		
Number uninfected after 11 weeks	2/20			0/20			6/20		
Pre-allergic deaths	2/18			1/20			1/14		
Weeks since infection	Type of Reaction								
	Positive	Intermediate	Combined	Positive	Intermediate	Combined	Positive	Intermediate	Combined
$1\frac{1}{2}$	0	0	0	0	0	0	0	0	0
$2\frac{1}{2}$	0	0	0	0	0	0	0	3	3/14
$3\frac{1}{2}$	0	0	0	0	0	0	0	4	4/14
$4\frac{1}{2}$	0	1	1/18	2	9	11/20	5	5	10/14
$5\frac{1}{2}$	1	8	9/18	8	9	17/20	10	3	13/14
$6\frac{1}{2}$	1	8	9/18	12	5	17/20	11	2	13/14
$7\frac{1}{2}$	8	7	15/18	17	1	18/20	12	1	13/14
$8\frac{1}{2}$	10	5	15/18	18	1	19/20	13	0	13/14
$9\frac{1}{2}$	12	3	15/18	19	0	19/20			
$10\frac{1}{2}$	13	3*	16/18						

Sixty mice inoculated intranasally with approximately 2 viable units. Tested at weekly intervals by inoculating 1:25 OT into metatarsal pads. Positive reactors show gross swelling of foot; intermediate reactors, any degree of swelling below this level. Combined reactors consist of all animals showing any degree of reaction.

* Three mice killed after 11 weeks showed gross lung involvement, but had not converted to a positive reaction.

to reversion of a proportion of the early reactors before conversion of the remainder was complete. From this time onward, reversion through the intermediate to the anergic state was common. It is important in relation to the view expressed here, i.e., that conversion coincides with the onset of specific resistance, to understand that the group of mice under consideration was not typical of other groups studied. For example, in mice on full diet inoculated intranasally with doses ranging from 2 to 500 viable units, the onset of anergy was slower so that there was a definite period, usually of several weeks' duration, in which pre-allergic deaths had ceased and post-allergic deaths had not begun.

Post-allergic deaths: Collected results from figure 2 and table 1 show that 23 mice died by the twenty-sixth week, all within two weeks of reverting to the anergic state. At the end of the experiment (thirty-four weeks), another 4 mice had died in the third, fourth, or fifth week after reversion. Thus, 85 per cent of the post-allergic deaths occurred within two weeks after reversion, and no mouse in this or any other experiment died while showing a positive footpad reaction. In one animal which showed incomplete reversion and loss of condition at the time of the last test, death occurred five days later, so that it is not definitely established that it was anergic at the time of death. Animals which died after reversion, unlike those which died in the early stages of infection, rarely showed more than 50 per cent of lung involvement, and sections stained with auramine showed no evidence of a proliferation of bacilli during anergy to account for their death.[4]

The results given in table 1 are of interest in their bearing on the relationship of persisting tuberculin allergy to survival and are similar to those of several groups of mice maintained on a full diet and observed during the later stages of infection by the intranasal route.

From the twentieth week onward some individual mice gave a negative tuberculin reaction for one or two weeks at a time. It was noted, however, that, unless there was a simultaneous deterioration in their clinical condition, later tests yielded intermediate or even positive reactions. It was clear also that at this stage of infection an intermediate reaction which lasted several weeks was associated with survival. That the incidence of intermediate reactors was probably not due simply to tuberculin saturation from repeated injections is shown by the proportion of positive reactions in mice Nos. 12 to 16 during the last six weeks of observation.

Cutaneous Reactions to Tuberculin in Guinea Pigs

In several experiments with guinea pigs inoculated intradermally, intramuscularly, or intranasally with doses ranging from 350 to 2×10^6 viable units, conversion to skin sensitivity took place in the third or fourth week after infection. Transitory intermediate reactions were seen in some animals during conversion, consisting of a small area of erythema and swelling, but there was no evidence

[4] This does not agree with the results obtained in post-allergic guinea pigs (Woodruff, C. E., and Kelly, R. G.: The correlation between anatomical changes and the allergic state in tuberculous guinea pigs, J. Immunol., 1942, *45*, 79). Confirmatory experiments are now in progress.

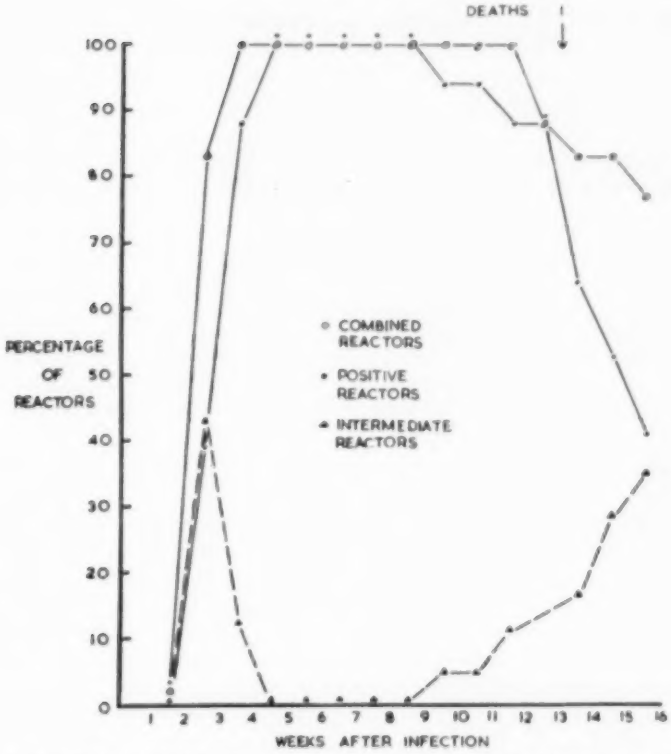

FIG. 3. Graph showing the progress of allergy in guinea pigs: 30 animals inoculated intranasally with 2,000 viable units were skin-tested weekly from the tenth day with a dilution of 1:50 OT. Ten control animals were consistently insensitive.

of the delay between conversion of the first and last animals characteristic of all groups of mice (*see* figure 2 and table 3).

In figure 3, a typical result, the conversion rate is recorded of 30 guinea pigs infected intranasally with approximately 2,000 viable units of H37Rv and kept on the same reduced diet as the mice. All animals had converted when tested four and one-half weeks later and this position was maintained until the tenth week, when some animals showed reversion to an intermediate reaction. In the thirteenth week, 4 animals became anergic and by the sixteenth week the number had risen to 7. At this stage one of these animals had died and another was seriously ill. Both showed very advanced disease at autopsy. When the remaining survivors were killed in the seventeenth week, all were found to have moderate to severe generalized tuberculosis and there was no gross pathologic distinction between positive, intermediate, and negative reactors.

Another group of 12 guinea pigs infected intranasally with 5,000 viable units

and kept on a full diet continued to give positive reactions from the fourth to the seventeenth weeks. At the time of writing (thirty-five weeks), 7 animals which reverted through intermediate to negative reactions without loss of condition subsequently gave an intermediate or positive reaction, reproducing the phenomenon seen in mice. Total lung involvement caused the death of 3 of these while showing a positive reaction. Five animals, showing uninterrupted reversion with emaciation, died within four weeks after the first negative reaction to tuberculin.

The Effect of Varying Experimental Conditions on the Conversion Rate of Mice

Age of mice at time of infection: Three groups of 20 mice aged, respectively, four, fourteen, and twenty-four weeks were infected intranasally with a suspension of H37Rv containing approximately two viable units per dose. At the conclusion of the experiment it was found that 8 animals had not become infected, reducing the numbers in the groups to 18, 20, and 14, respectively. Four animals which died without developing a footpad reaction appear in the results as having failed to convert. The results of weekly tuberculin tests are set out in table 2.

Regardless of whether the result is assessed in terms of positive reactors alone or of combined reactors, the average conversion rate was much faster in the older groups. At nine and one-half weeks, 3 of the young group had not advanced beyond an intermediate reaction and one was still negative, in contrast to the full conversion of the mice still alive in both older groups. At the end of the experiment a week later, 3 intermediate reactors remained in the youngest group.

Alternate animals were killed as soon as they showed a full positive reaction to tuberculin in order to determine the degree of lung involvement. These were carried on subsequently as positive reactors. The rate of progressive lung consolidation, which was readily observed because of the very small infecting dose used in this experiment, was much the same in all three groups. Therefore, animals converting early did so when lung involvement was minimal. For example, the first mice to convert in the fourteen- and twenty-four-week groups had only 1, 2, or 3 visible lung tubercles averaging 0.5 mm. in diameter. Those which converted late showed progressively greater lung consolidation. The most seriously affected lungs, showing approximately 30 per cent of consolidation, were those of the 3 remaining intermediate reactors of the youngest group when they were killed in the eleventh week.

Sex of mice: During recent experiments, whatever the strain of organism, dose, or route of inoculation used, approximately equal numbers of mice of both sexes and of the same age have been included. No significant difference was observed in the rate of conversion, nor is there any obvious reason for expecting one.

Virulence, viability, and dose of infecting strain: Because the influence of age was not appreciated until recently, no check was made in the early stages of the precise age of the mice at the time of inoculation, although the average age was usually three or four months. Therefore, no importance is attached to the relative tuberculin conversion rates of the groups in this series, and available results are submitted merely for the evidence they provide that conversion may also

TABLE 3

THE ALLERGIC RESPONSE OF MICE AGED THREE TO FOUR MONTHS TO LIVING AND DEAD H37Rv AND TO BCG

Strain	State	Approximate Dose of Bacilli	Route of Inoculation	Number of Mice		Percentage of (*a*) Positive and (*b*) Combined Reactors at Weekly Intervals								
						2½	3½	4½	5½	6½	7½	8½	9½	10½
H37Rv	Living	2	I/N	19*	*a*	0	0	11	42	66	89	95	100	100
					b	0	0	58	89	92	95	100		
H37Rv	Living	80	I/N	64	*a*	0	0	46	55	62	68	92	100	85
					b	0	22	85	82	87	89	96	100	95
H37Rv	Living	450	I/N	102	*a*	0	27	60	71	82	95	100	—	—
					b	9	53	73	86	96	100		—	—
H37Rv	Living	3,000	I/D	20	*a*	0	0	0	0	20	30	30	50	—
					b	0	10	30	40	80	80	80	90	—
H37Rv	Killed	0.5 mg.	I/N	10	*a*	0	0	0	0	0	0	0	0	0
					b	0	20	10	30	30	90	90	50	30
H37Rv	Killed	5.0 mg.	I/P	10	*a*	0	0	0	40	70	90	100	100	100
					b	0	0	60	100					
BCG	Living	2×10^6	I/P	45	*a*	0	0	18	55	71	92	100	100	89
					b	12	55	73	90	100				100

Routes of inoculation—I/N = intranasal; I/D = intradermal (footpad); I/P = intraperitoneal; – = not tested.

* In this group, alternate animals were killed at conversion (*see* table 2) and were subsequently carried on as positive reactors. Therefore the figure of 100 per cent of positive reactors at nine and one half weeks is presumptive.

follow the inoculation of avirulent, or even killed virulent, organisms and that more than one route may be effective.

Mice infected intranasally with doses of 2, 80, or 450 viable units of H37Rv had all converted to fully positive tuberculin reactions by about the ninth week. To assume that the observed shift of reactors in the highest dose group one week to the left in the table was due to the larger dose would not be justified in the absence of precise knowledge of the respective ages of the groups (*see* table 2). It is obvious that the effect of still larger doses, given by different routes, on mice of the same age is yet to be determined. Living H37Rv organisms when given intradermally, i.e., by footpad inoculation, in a dose of 3,000 viable units produced a fairly typical response in terms of combined reactors, but the emergence of positive reactions was undoubtedly slow. Tests on this group were discontinued at nine and one-half weeks. Killed tubercle bacilli (H37Rv) in a relatively very large intranasal dose (0.5 mg.) produced no positive reactors within ten and one-half weeks of inoculation, but intermediate reactions were shown by the majority after seven and one-half weeks. This is interpreted as indicating that even large doses of killed bacilli by this route may provide only a relatively poor allergic stimulus. On the other hand, a tenfold larger dose of killed organisms (5.0 mg.) given intraperitoneally was followed by rapid, full conversion and the reaction was strongly maintained for several weeks. BCG (2×10^6 viable units

intraperitoneally) also provided a strong stimulus of similar intensity to that of the killed organisms inoculated intraperitoneally.

Adrenalectomy: Twenty-eight mice were adrenalectomized and, on the tenth day, 10 survivors which appeared to be fully recovered, together with 10 control animals of the same age (four months), were infected intranasally with approximately 900 viable units of H37Rv. Three of the adrenalectomized mice died during the next three days.

The tuberculin conversion rate of both groups ran a parallel course, so that after four and one-half weeks the control animals included 3 positive and 7 intermediate reactors, while the adrenalectomized group showed 2 positive and 5 intermediate reactors. Adrenalectomy, therefore, had not been responsible for either a more rapid conversion to the allergic state or a stronger individual sensitivity to tuberculin. The animals were then killed and it was confirmed that no adrenal tissue remained. Neither macroscopically nor microscopically did it appear that the operative procedure had exerted any influence on the disease in these mice during the period of observation. This was intended as a pilot experiment, but, in view of the results, repetition on a larger scale seemed unnecessary.

Tuberculin Shock

It was an obvious corollary to skin sensitivity that mice would prove susceptible to tuberculin shock if examined under the right conditions. The possibility of deaths being an expression of an anaphylactic rather than an allergic reaction of the tuberculin type had to be excluded. The criteria adopted, therefore, were that the reaction should be of the delayed type and associated with

TABLE 4

THE DEVELOPMENT OF SENSITIVITY TO INTRAPERITONEAL OLD TUBERCULIN IN TUBERCULOUS MICE

Number of Animals	Inoculum	Dose (Viable Units)	Route of Infection	Interval Since Infection	Positive Footpad Reaction	Dose of OT I.P	Specific Deaths (24–48 Hours)	Sublethal Shock
				weeks		*ml.*		
50	None (controls)	None	—	—	0/50	0.2	0/50	0/50
10	H37Rv	500	I/N	4	Not done	0.2	0/10	0/10
10	BCG	55,000	I/P	4	Not done	0.2	0/10	0/10
10	H37Rv killed	0.5 mg.	I/P	4	Not done	0.2	0/10	0/10
4	H37Rv	450	I/N	7	4/4	0.1	0/4	0/4
8	H37Rv	450	I/N	8	8/8	0.2	3/8	0/8
13	H37Rv	80	I/N	8	13/13	0.1	2/13	2/13
13	H37Rv	80	I/N	8	13/13	0.2	2/13	4/13
8	H37Rv	450	I/N	9	8/8	0.2	6/8	2/8
8	H37Rv	450	I/N	10	8/8	0.2	8/8	0/8
10	H37Rv	80	I/N	10	10/10	0.2	7/10	1/10
7	H37Rv	25	I/N	14	*5/7	0.2	7/7	0/7

Routes of inoculation; I/P = intraperitoneal, I/N = intranasal.

* Two mice were anergic to skin test, but sensitive to tuberculin shock. Uninfected control animals were skin-tested weekly and a group of 4 or 5 subjected to tuberculin shock whenever infected animals were tested.

the acute hemorrhagic infiltration of lesions accepted as typical of tuberculin shock in guinea pigs.

Having shown that normal mice were quite insensitive to single doses of up to 0.75 ml. of OT intraperitoneally or to repeated weekly injections of 0.2 ml., selected groups of infected animals were challenged together with an equal number of controls, usually with 0.2 ml. doses. The results are set forth in table 4.

Mice inoculated with living or dead tubercle bacilli of the strain H37Rv or with BCG were insensitive to tuberculin shock after four weeks. When tests on animals infected intranasally with H37Rv were continued for a longer period, some deaths resulted at the eighth week. After nine weeks most animals either died or recovered after a severe reaction, but even then the result could not be predicted with certainty. Except for the first three groups of infected animals in the table, all received weekly footpad injections of a dilution of 1:25 OT, which could well have exercised a partial desensitizing action. As no infected controls that had not been regularly tested were available for the present series, this must remain an unanswered question. However, the results of inoculating 0.2 ml. of OT intraperitoneally into infected guinea pigs which were receiving weekly skin tests suggested that the skin tests may have exerted some desensitizing action in this species or that the dose was inadequate. In groups of four guinea pigs tested between the fourth and twelfth weeks after infection, there was seldom more than one fatal reaction, although most of the animals suffered from a sublethal shock which lasted two or three days.

The Pathogenesis of Tuberculous Lesions

Previously reported studies (1, 2, 13) focussed attention on the mouse as an experimental tool in tuberculosis research because of the rapid multiplication of stainable bacilli in the lungs. The small numbers of bacilli detectable in guinea pig lesions suggested that a comparison of mouse lung with guinea pig lung might confirm this apparent difference and help in understanding the reaction of the mouse to tuberculosis. The experiments reported here were carried out prior to the discovery of tuberculin sensitivity in the mouse. Hence, it is not possible to relate the changes in bacillary numbers and in the cellular pattern of individual lesions mentioned here to the allergic state other than as a series of changes taking place over the same period. Therefore, these results will be reported only briefly for the evidence they provide of a specific cellular change which appeared concurrently with a smaller number of stainable bacilli during the period shown to be that of conversion to the allergic state.

Mice inoculated intranasally with approximately 350 viable units of H37Rv were compared with guinea pigs, half of which were inoculated with 350 units and the remainder, on a body-weight basis, with twenty times that dose. As the observed effect of the larger dose was to increase the number of lung tubercles, but not the progress of individual lesions, no further distinction will be made between these two groups of guinea pigs except in relation to lung counts. The experiment involved 120 mice and 60 guinea pigs, the former being killed in groups of 6 over a period of nineteen weeks and the latter in groups of 4 over fifteen weeks.

TABLE 5

CELLULAR CHANGES IN MOUSE LUNG RELATED TO STAINABLE AND VIABLE BACILLI (Guinea Pig Included for Comparison)

Time in Weeks	Stainable Bacilli in Lung Lesions (F. M.)		Number of Colonies from the Lung $\times 10^3$		Cellular Changes in Mice		
	Mice	Guinea Pigs	Mice Dose* 350	Guinea Pigs Dose: 7,000	Macrophage Concentration	Focal Necrosis	Granulocytic Infiltration
1	+	0	3†	9	−	−	+
2	++	0	115	846	+	+	+
3	+++	+	520	33	++	+	+
4	++++	++	720	1,440	+++	+	+
5	>++++	++	1,400	17,000	++++	++	+
6	+++	++	91	5,400	>++++	++	+
7	++	++	505	187	>++++	++	++
8	++	++	360	3,750	>++++	+++	++
10	++	+	1,400	330	>++++	+++	+++
13	++	+	1,520	54	++++	+++	+++

F. M.—sections stained by fluorescence microscopy (figures 4–13).

* Infective dose by intranasal inoculation: Mice 350 viable units, guinea pigs 7,000 viable units of H37Rv. Dose assessed on body weight.

† The figures in the table $\times 10^3$ are calculated from the number of bacilli per half-lung recoverable by surface plating. The other half of each lung was used for section. Each result is the average of 4 mice or 2 guinea pigs—triplicate platings.

Cellular changes in mice: + to >++++ have been used to indicate approximate degree of each response.

The results in table 5 relate the cellular changes observed in mouse lung to the stainable and viable organisms present. The guinea pig figures are included for comparison, although it should be noted that the infecting dose in these animals was the larger one, viz., 7,000 viable units or twenty times the mouse-infecting dose.

Of the classical components of tubercles, only foamy macrophages of epithelioid type and focal necrosis were seen. The macrophages increased in numbers, filling the alveoli and bronchioles, and were the predominant cell type from approximately the sixth week onwards. There was a gradual increase in focal necrosis of the macrophages within the alveoli which was associated with polygranulocytic invasion, and after the eighteenth week some restoration of alveoli appeared to be taking place. An interesting feature of the lesions was the absence of giant cells, fibrosis, caseation and calcification, and the maintenance of the blood supply to the alveoli.

The rate of multiplication of stainable bacilli was much faster in the mouse and, whereas the numbers in the guinea pig became relatively stable, those in the mouse suggested a rather dramatic reduction from approximately the sixth week onward (*see* figures 4–13). This result does not agree with the number of colonies isolated from the mouse lung for, although these showed a slight decrease during the six- to eight-week period, corresponding to the marked fall in stainable bacilli, they rose in the final weeks when stainable bacilli were relatively scanty. This discrepancy has yet to be investigated.

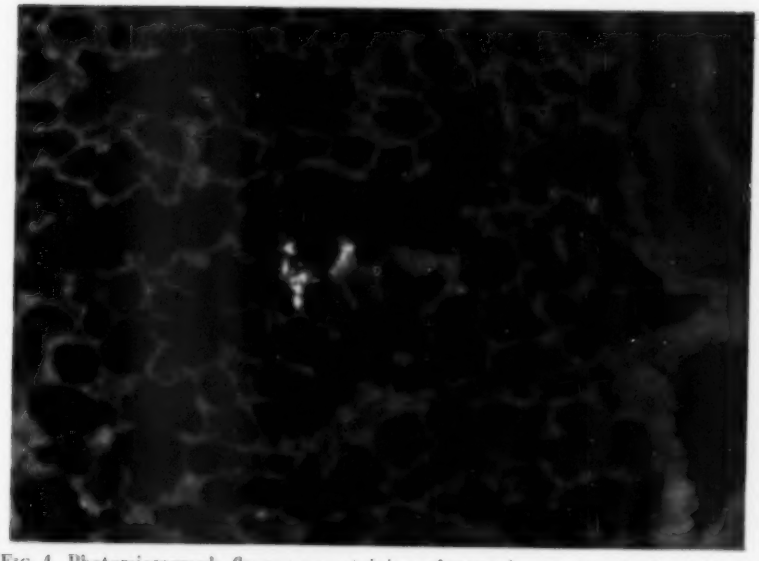

FIG. 4. Photomicrograph, fluorescence staining, of mouse lung one week after intranasal inoculation showing a clump of organisms and slight septal thickening. (×400)

FIG. 5. Photomicrograph, fluorescence staining, of guinea pig lung one week after intranasal inoculation. No bacilli are visible but consolidation is already well advanced. (×100)

FIG. 6. Photomicrograph, fluorescence staining, of mouse lung after three weeks, showing numerous organisms. (×100)

FIG. 7. Photomicrograph, fluorescence staining, of guinea pig lung after three weeks. A few organisms are present in a necrotic focus. (×100)

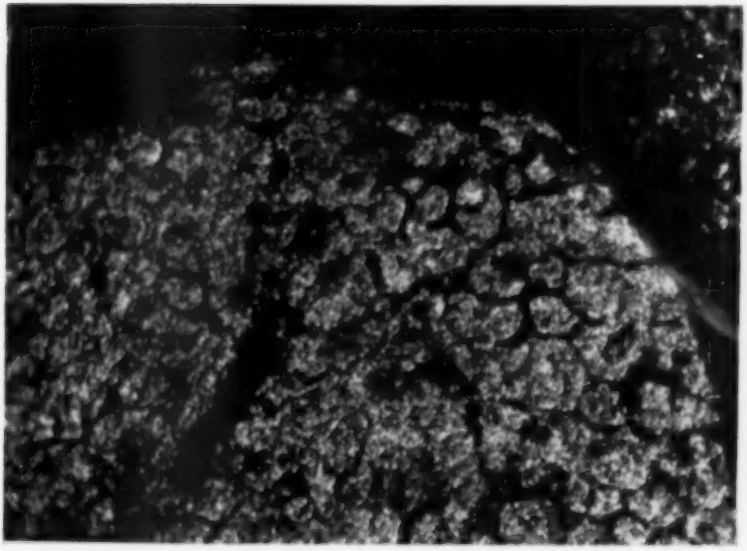

FIG. 8. Photomicrograph, fluorescence staining, of mouse lung after five weeks. Great numbers of organisms are present in the macrophages filling the alveoli. The outlines of the intact alveolar walls are visible by contrast. (×100)

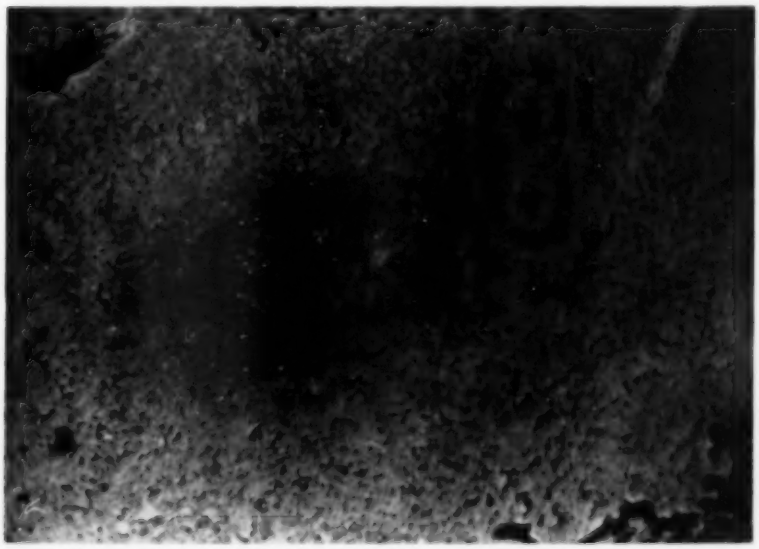

FIG. 9. Photomicrograph, fluorescence staining, of guinea pig lung after five weeks. The caseous center of a tubercle contains a few organisms; peripheral fibrosis has destroyed alveolar structure. (×100)

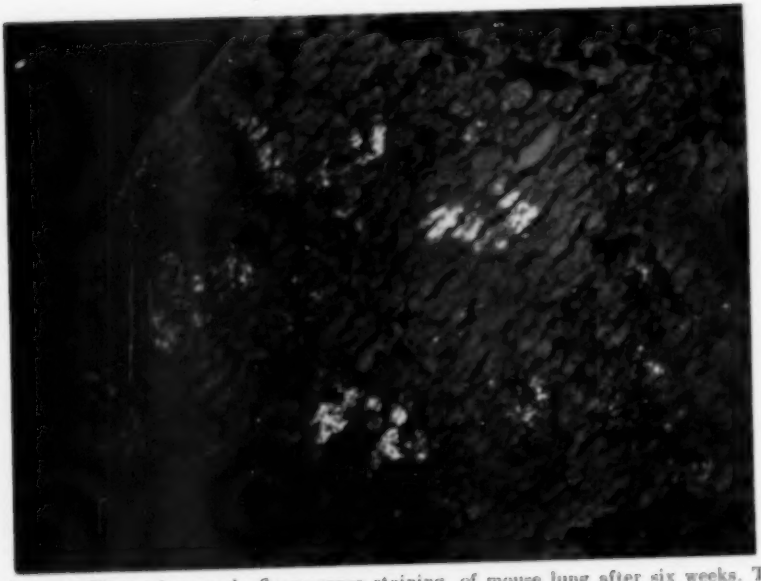

FIG. 10. Photomicrograph, fluorescence staining, of mouse lung after six weeks. The organisms are much reduced in number and are distributed in isolated groups. (×100)

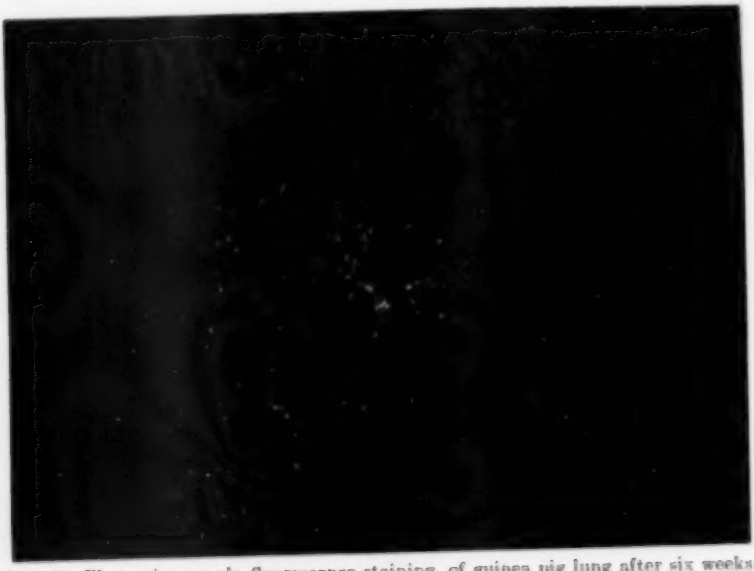

FIG. 11. Photomicrograph, fluorescence staining, of guinea pig lung after six weeks. A few organisms are present in the caseous focus. (×100)

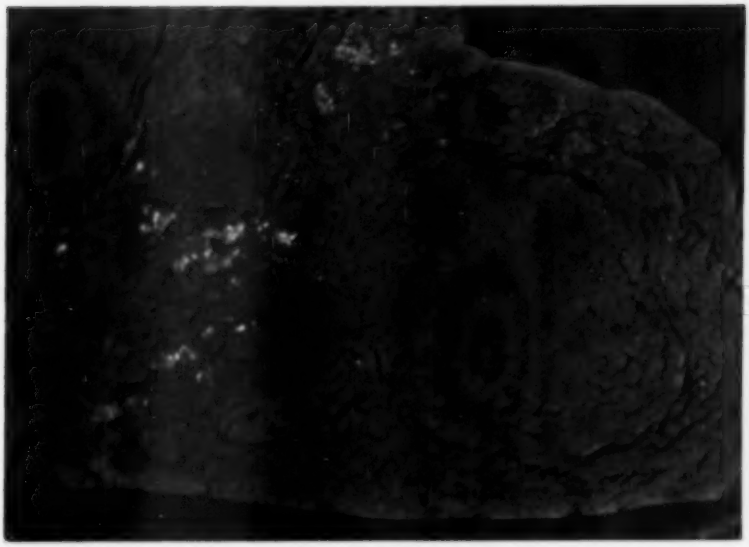

FIG. 12. Photomicrograph, fluorescence staining, of mouse lung after thirteen weeks. A few clumps of organisms are still present. (×100)

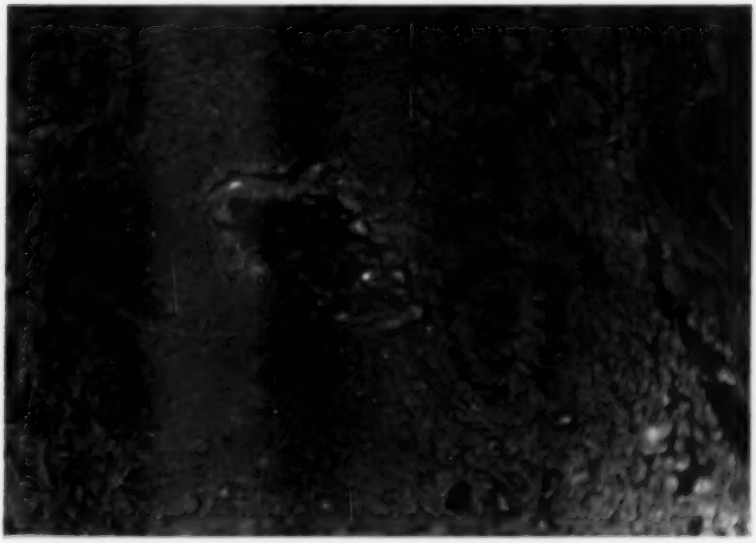

FIG. 13. Photomicrograph, fluorescence staining, of guinea pig lung after thirteen weeks. Organisms are present in the caseous focus and in the surrounding fibrous capsule. (×100)

The temporary reduction in the viable number of bacilli in guinea pig lungs at three weeks (only 2 animals were used, but comparable counts were obtained) may have been associated with the onset of allergy, for it was found in skin lesions produced by intradermal inoculation that a decrease in the number of stainable bacilli occurred after tuberculin conversion (unpublished). The evidence suggests that the apparent reduction in stainable and in viable bacilli in the mouse and the aggregation of macrophages were both related to the onset of allergy in this species and to the beginning of a period of increased resistance to the fatal effects of the disease.[5]

DISCUSSION

The development and progress of allergy in tuberculous mice were compared with that in guinea pigs. Old Tuberculin diluted 1:25 was used for routine testing of mice, although sufficient tests were done with the equivalent strength of PPD to be satisfied that PPD produced a similar response. In terms of skin sensitivity and lethal shock, the two species showed the same fundamental response although there were minor points of difference. Under weekly tests individual guinea pigs usually converted abruptly from a negative to a positive skin reaction, with only an occasional intermediate reaction. In contrast, individual mice almost invariably showed an intermediate reaction lasting one or two weeks before giving a positive reaction. Individual guinea pigs all converted within two weeks of one another and there were no preconversion deaths, whereas the interval between conversion of the first and last members of a group of mice was occasionally four but usually five or six weeks, and preconversion deaths occurred. No explanation of this wide scatter in mouse tuberculin conversion times can be offered at present, but it appears to be unrelated to possible cross-infection, as it occurred whether living or dead organisms were used.

Conversion of mice to the allergic state was associated with the onset of a period of increased resistance. The lung lesions were characterized by a cellular pattern lacking many of the elements of the classical tubercle. No fibrosis, caseation, or calcification were noted, and the lesions remained vascularized. The outstanding cellular change was the accumulation of dense masses of foamy macrophages of the epithelioid type in the alveoli, bronchioles, and bronchi. In the later stages when these masses of cells became necrotic and were removed by phagocytosis, there was some evidence of restoration of function in previously consolidated areas. The apparent simplicity of this cellular response encourages the conclusion that the cellular defense of the mouse is based on these masses of macrophages and that their appearance is intimately concerned with the onset of allergy.

Mice either died without converting or remained in good physical condition as long as footpad tuberculin sensitivity was present. This period was rarely as

[5] Further evidence of the association of uninhibited multiplication of bacilli with the absence of allergy is provided by the report of Woodruff and Willis (Allergy and desensitization in experimental tuberculosis, J. Immunol., 1939, *37*, 549). Tuberculous guinea pigs in which allergy was suppressed showed a typical mouse-type infection.

short as four weeks and occasionally as long as thirty weeks. Temporary loss of cutaneous sensitivity was noted in some animals which had been infected for long periods, but the majority of mice became anergic and obviously ill at the same time and died soon afterward. Eleven guinea pigs which died of tuberculosis reverted and remained anergic to tuberculin for one to four weeks before death. The physical deterioration first appeared in the guinea pigs at the onset of anergy as it did in mice. Seven animals in a recent experiment also showed the temporary anergy without loss of condition seen in mice. Total lung involvement caused the death of 3 of these animals while showing a positive reaction.

A large dose of Old Tuberculin by the intraperitoneal route killed a small proportion of mice tested eight weeks after infection and most of those tested from the ninth week onward. That this was an allergic phenomenon and not anaphylactic seems certain in view of the delayed death and the intense hemorrhagic congestion of the lung lesions that were constant features. Normal mice showed no ill-effects from repeated dosage with 0.2 ml. or even larger amounts of tuberculin. It is concluded that, when the allergic state is firmly established, the majority of mice can be killed by tuberculin shock. Whether the omission of weekly skin tests would cause an earlier fatal reaction has not been determined, but the same dose of tuberculin killed only a small proportion of infected guinea pigs which had been subjected to weekly skin tests. On a weight basis, the mice received approximately forty times the skin-test dose and twenty times the intraperitoneal dose of tuberculin given to guinea pigs.

Of a number of factors possibly influencing the rate of mouse conversion which have been studied, the age of the mice at the time of infection was most important. Not only was the average conversion rate of very young animals slower than that of young adults or older mice but, as a result, a larger average degree of lung involvement was present at the time of tuberculin conversion in the young ones. This suggests that the slower conversion of young animals was due, not to the slower formation of lesions, but to other factors; notably the less effective resistance they showed to infection. Insufficient results are yet available to assess with any accuracy the effect of dosage and route of inoculation on conversion rates.

Adrenalectomy performed ten days before intranasal infection apparently failed to affect the rate of conversion or the intensity of the footpad response. The number of stainable bacilli and the degree of lung involvement were not discernibly different from those factors in control mice of the same age.

In C57 mice with a primary intranasal infection there was an initial rapid multiplication of tubercle bacilli leading to an accumulation of densely packed organisms in the lesions, reaching a peak of numbers by approximately the fifth week. Animals killed in the later stages showed only small scattered clumps of stainable bacilli. In guinea pigs inoculated by the same route, multiplication was very slow. Stainable bacilli were not detected before the third week and from the fourth week onwards remained fairly constant.

The question may be asked whether the experimental conditions permit the conclusion to be drawn that the sequence of events in all infected mice was a

multiplication of bacilli, followed by a reduction in numbers. It is true that the picture presented has been arrived at by observing a large number of mice which died or were killed at regular intervals after infection. Those which died in the preconversion period showed almost completely consolidated lungs packed with bacilli, but mice examined during the period corresponding to allergy or anergy showed only partial lung consolidation and small, scattered clumps of bacilli. It could be argued that the bacillary numbers in these mice which survived the early stages of infection may never have exceeded the numbers found at the time they were killed or died and, therefore, that acquired resistance was not necessarily associated with the actual destruction of bacilli.

The evidence from counting the viable numbers in lungs does little to clarify the situation. The present results agree with those of Pierce, Dubos, and Schaefer (15) and bear no apparent relationship to the stainable bacilli. A comparison of the results in mouse and guinea pig lungs suggests that the discrepancy is considerable. Regardless of the difference in the infecting dose (table 5), stainable numbers in these two animals cannot be reconciled with viable counts from the lungs unless it is supposed that the bacillary death rate in the mouse lags only slightly behind the multiplication rate and that many of the stainable bacilli are dead. Another possible explanation would be that the increase in viable bacilli in the late stages of infection, with no detectable increase in stainable organisms, is related to the presence of nonacid-fast forms. This is to be investigated.

Many of the questions raised by the present findings require further study. Mackaness (16) believes that there is a relationship between the density of macrophages in tissue cultures and their bacteriostatic activity. Such a relationship could well exist in the mouse lung and, reinforced by the little-understood action of allergy, be responsible for the actual destruction of bacilli. There is already evidence, in the ability of macrophages to confer passive tuberculin sensitivity on normal animals, of their intimate association with allergy. This relationship may be clarified by further study of the response of an animal which fails to produce other cellular components of the classical tubercle.

It is yet to be explained why, in a group of mice with only moderate lung involvement and no gross lesions elsewhere in the body, some mice suddenly become anergic and die. The stainable bacilli in their lesions do not increase at this stage and it is yet to be shown whether "L" forms lacking acid-fastness are responsible or whether toxic breakdown products of the inflammatory process or of the bacilli play a part in the fatal result.

In conclusion, the present findings require a further revision of the concept that mice differ greatly from other animals in their response to tuberculosis. Allergy does develop in mice when they are exposed by a suitable route to living or dead virulent bacilli or to BCG. With living organisms given intranasally, its onset corresponded to a period of dense aggregation of macrophages in lung lesions, and while allergy persisted the animals did not die of tuberculosis. Whether or not an intermediate footpad reaction is indicative of the same degree of resistance to the progress of the disease as a fully positive reaction is not yet certain.

The degree of resistance to secondary infection associated with these two stages during tuberculin conversion may be determined in the course of immunization experiments with BCG and killed H37Rv now in progress. It has been shown in the present results that an intermediate reaction over several weeks in long-standing cases of primary infection is compatible with a stable clinical condition in mice and that this reaction does not appear to stem from tuberculin saturation due to regular testing.

SUMMARY

C57 black mice do become allergic when exposed to tubercle bacilli. The allergic state is indicated by swelling of the foot after the local injection of tuberculin or by death following intraperitoneal injection.

A complete reaction to tuberculin usually develops between the fourth and ninth weeks after infection and allergy persists for periods ranging from four weeks to more than thirty weeks.

Animals may die of tuberculosis before tuberculin conversion or after reversion to the anergic state as determined by footpad tests.

Death is associated with anergy but not with an increase in the number of stainable bacilli in the lesions, despite the fact that viable counts show a definite increase in the number of bacilli in the lungs in the terminal stages.

Allergy appears to follow the accumulation of macrophages in the lung lesions, although the precise relationship of these cells to the phenomenon of enhanced resistance and to the subsequent concurrent loss of allergy and resistance is yet to be determined. Using young mice and small intranasal infecting doses to obtain a delayed onset of specific resistance, a period of several weeks is available for studying the changes which result in the transition from natural to acquired resistance.

SUMARIO

La Alergia en la Tuberculosis Murina Experimental

Los ratones negros de la cepa C57 se vuelven alérgicos cuando se les expone a bacilos tuberculosos. El estado alérgico queda indicado por el edema de los pies después de la inyección local de tuberculina o por la muerte consecutivamente a la inyección intraperitoneal.

El pleno viraje suele presentarse entre la cuarta y la novena semanas consecutivas a la infección y la alergia persiste durante períodos que varían de cuatro a más de treinta semanas.

Los animales pueden fallecer de tuberculosis antes del viraje o después de volver al estado anérgico, determinado esto por medio de pruebas podales.

La muerte se asocia con anergia, pero no con aumento del número de bacilos colorables en las lesiones, a pesar de que las numeraciones de los viables revelan au aumento bien definido del número de bacilos en los pulmones en las etapas terminales.

La alergia parece seguir a la acumulación de macrófagos en las lesiones pulmonares, aunque está aun por determinar la precisa relación de esos glóbulos con el fenómeno de la mayor resistencia y la subsiguiente pérdida concomitante de la alergia y la resistencia. Con el uso de ratones jóvenes y de pequenas dosis infectantes intranasalmente, se cuenta con un período de varias semanas para estudiar las alteraciones que culminan en la transición de la resistencia natural a la adquirida.

RESUME

Allergie dans la tuberculose expérimentale de la souris

Des souris noires de la souche C 57 deviennent toujours allergiques lorsqu'elles sont exposées aux bacilles tuberculeux. L'état allergique est indiqué par le gonflement de la patte, à la suite de l'injection locale de tuberculine, ou par la mort quand l'injection est intrapéritoniale.

Le virage complet se produit habituellement entre la quatrième et la cinquième semaine qui suit l'inoculation; cette allergie persiste pendant des périodes allant de quatre à plus de trente semaines.

Les animaux peuvent mourir de tuberculose avant le virage ou après le retour à l'état anergique démontré par le test épidermique au niveau de la patte.

La mort est associée à l'anergie et non à l'accroissement du nombre de bacilles colorables dans les lésions, en dépit du fait qu'au stade terminal la numération de la population viable révèle un accroissement défini du nombre des bacilles dans le poumon.

L'allergie paraît succéder à l'accumulation des macrophages dans les lésions pulmonaires, bien que la relation précise entre ces cellules et le phénomène de résistance accrue et la perte concomitante ultérieure de l'allergie et de la résistance reste à déterminer. Quand l'inoculation est pratiquée sur de jeunes souris avec des doses intra-nasales faibles, qui permettent d'obtenir un retard dans l'apparition de la résistance spécifique, on dispose d'une période de plusieurs semaines où il est possible d'étudier les modifications qui résultent de la transition entre la résistance naturelle et la résistance acquise.

Acknowledgments

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THE INACTIVATION OF ISONIAZID BY FILTRATES AND EXTRACTS OF MYCOBACTERIA[1, 2]

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INTRODUCTION

Previous *in vivo* studies using mice have shown that the suppressive action of isoniazid is inversely proportional to the size of the infecting dose of *Mycobacterium tuberculosis* var. *hominis* (H37Rv) (1). The results of these mouse studies suggested that virulent tubercle bacilli (H37Rv) might produce and possibly liberate into their environment a substance which would reverse the tuberculostatic action of isoniazid.

Further studies were done in which the *in vitro* bacteriostatic activity of isoniazid was determined when using inocula containing different numbers of organisms of the H37Rv strain. It was found that the growth of very large inocula was inhibited much less by isoniazid, again suggesting that large numbers of organisms might be able to withstand the deleterious effect of isoniazid by producing some substance which would inactivate this compound. This same relationship has been noted between penicillin and penicillinase-producing strains of staphylococci (2–4).

The present report covers in detail experiments designed to show the relationship between the size of the inoculum and the bacteriostatic action of isoniazid. In addition, experimental results are presented which show that an "anti-isoniazid" substance is produced by several species of mycobacteria and one strain of *Escherichia coli*.

MATERIALS AND METHODS

Strains: Since the H37Rv strain multiplies very slowly, the isoniazid-susceptible saprophytic fast-growing strain, 607, was employed as the test organism to determine the inactivation of isoniazid. In later experiments the strains H37Rv and *M. phlei* were also used to check the results obtained with the 607 strain. Filtrates were obtained from cultures of strains 607, H37Rv, H37Ra, *M. phlei*, H37Rv made resistant *in vitro* to 5.0 mg. of isoniazid per 100 ml. (H37RvR-isoniazid) and *E. coli*.

Suspensions of mycobacteria used as inocula for the bacteriostatic tests were prepared and standardized as previously described (5).

Preparation of isoniazid dilutions: The various concentrations of isoniazid were prepared by making twofold dilutions in a modified Proskauer and Beck medium (5) from a 100.0 mg. per 100 ml. solution. The first volume of each dilution was 5.0 ml., and sterilization was carried out in the autoclave, using a pressure of 15 pounds for twenty minutes. All sets of isoniazid dilutions were used within three or four days after preparation, since isoniazid tends to break down in this medium upon prolonged standing (three weeks) at room temperature.

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This study was aided, in part, by a grant from Parke, Davis & Company, Detroit, Michigan.

Preparation of filtrates: Filtrates of cultures of 607 were used in the earlier experiments, and the findings were used as a guide for the testing of filtrates of cultures of H37Rv, H37Ra, *M. phlei*, H37RvR-isoniazid, and *E. coli* after it had been determined that the "anti-isoniazid" activity was the same in filtrates as in the supernatants of centrifuged cultures. All filtrates were prepared by filtering the cultures through either Mandler or ultra-fine sintered glass filters. The pH of the filtrates was measured with a Beckman electrometer. Unless otherwise noted, 1.0 ml. of the sterile filtrate was added aseptically to each dilution of isoniazid, the tubes were inoculated with 10^{-3} mg. of the test organism, and were incubated at 37°C. The tubes were inspected daily for six days to determine the bacteriostatic end point when the 607 and *M. phlei* strains were used as test organisms, and were inspected weekly for three weeks when the H37Rv strain was employed.

Preparation of cell extracts: Eight-day cultures of strain 607 and thirty-day cultures of strain H37Ra were filtered separately through coarse sintered glass filters and washed with large volumes of double distilled water. The cells were then placed in a 200 ml. centrifuge bottle and mixed with glass powder and enough 0.01 M phosphate buffer, pH 7.0, to make a thick paste. Steel balls were added to fill one-fourth of the bottle, and the cells were ground for sixteen to eighteen hours at 4°C., using a ball mill housed in a refrigerator. After adding a small amount of buffer and removing the steel balls, the mixture was centrifuged at 5,000 r.p.m. for one hour in a Spinco Model L ultracentrifuge, the supernatant was removed and centrifuged again at 10,000 r.p.m. for fifteen minutes. The supernatant from this centrifugation was used to add to the isoniazid dilutions.

RESULTS

The effect of the size of the inoculum of three strains of mycobacteria, H37Rv, 607, and *M. phlei*, on the bacteriostatic activity of isoniazid is presented in table 1. The two largest inocula of each of the organisms were much less susceptible to isoniazid. The organisms (H37Rv) of each inoculum growing in the highest concentration of isoniazid were subcultured and their susceptibility to isoniazid was determined. They were found to be as susceptible as the original inoculum.

In table 2 are listed the results obtained when filtrates taken from cultures of 607 and H37Rv of different ages were added to dilutions of isoniazid. The values in the table represent the ratio between the least amount of isoniazid which in the control series of tubes completely inhibited growth and the least

TABLE 1

THE EFFECT OF THE AMOUNT OF INOCULUM ON THE TUBERCULOSTATIC ACTIVITY OF ISONIAZID

Amount of Inoculum	Least Amount of Isoniazid in Mg. per 100 Ml. Which Completely Inhibited Growth		
	Strain of *Mycobacterium*		
	607	H37Rv	*Phlei*
mg.			
1.0	50.0	12.5	50.0
10^{-1}	0.78–3.10	0.019	6.2
10^{-2}	0.19–0.04	0.0045	0.02
10^{-3}	0.09–0.04	0.0045	0.01
10^{-4}	0.02	0.0045	0.005
10^{-5}	0.02	0.0045	0.0006
10^{-6}	0.02	0.0022	No growth

TABLE 2

THE EFFECT OF FILTRATES TAKEN FROM CULTURES OF M. 607 AND H37Rv AFTER DIFFERENT PERIODS OF INCUBATION ON THE BACTERIOSTATIC ACTIVITY OF ISONIAZID FOR M. 607

Culture of *Mycobacterium*			
H37Rv		607	
Period of Incubation in Days of H37Rv Cultures	Fold Decrease in Isoniazid Activity Produced by H37Rv Filtrate	Period of Incubation in Days of 607 Cultures	Fold Decrease in Isoniazid Activity Produced by 607 Filtrate
7	0	3	0
14	2	6	8
21	16	9	16
35	32–64	19	16
49	32–128	22	32–64
53	64–256	26	128
62	32	27	32–512
67	16	28	128
81	4	34	128
85	8	39	32
95	2	40	64–256
102	2	57	16
		74	4

amount of isoniazid which completely inhibited growth in the tubes to which filtrate had been added. The least amount of isoniazid needed to inhibit growth in the isoniazid series of tubes to which filtrate had been added was divided by the least amount of isoniazid needed to inhibit growth in the control isoniazid series. This gives a value which represents the fold decrease in the bacteriostatic activity of isoniazid produced by the addition of the filtrate. The fold decrease as given in the table was determined from the bacteriostatic end points obtained on either the third or fourth day of incubation.

With increase in age of the cultures, progressively more "anti-isoniazid" substance was released until a certain time, approximately four weeks with the 607 strain and eight weeks with H37Rv. Thereafter, the amount of "anti-isoniazid" substance gradually decreased. Experiments performed with filtrates of cultures of *M. phlei* and H37Ra gave similar results; a 64 to 128 fold decrease in approximately four weeks with *M. phlei* filtrates and a 32 to 128 fold decrease in four to eight weeks with H37Ra filtrates. As 607 and *M. phlei* multiply at a much faster rate than H37Rv and H37Ra, one would expect the highest concentration of the "anti-isoniazid" substance to appear at an earlier time than it would in cultures of H37Rv and H37Ra.

The filtrates obtained from cultures of the isoniazid-resistant strain did not antagonize the bacteriostatic action of isoniazid to as great a degree as the filtrates obtained from cultures of the isoniazid-susceptible strains. There was a 4 to 8 fold reduction in bacteriostatic activity of isoniazid with 7 filtrates of cultures which varied in age between thirty-five and ninety-nine days.

Similar experiments were conducted using filtrates from cultures of *E. coli*, an organism which is normally highly resistant to isoniazid since a concentration of 25.0 mg. or more per 100 ml. is needed to inhibit growth. Filtrates obtained from cultures of this organism which had been grown in Proskauer and Beck medium and varied in age between three and twenty-eight days decreased the bacteriostatic activity of isoniazid for the 607 strain from 16 to 64 fold. The oldest filtrates were only slightly more active than the youngest.

The decrease in isoniazid activity on adding varying amounts of the filtrates to the isoniazid dilutions is shown in table 3. The amount of isoniazid which completely inhibited growth depended upon the amount of filtrate which was added, since 0.1 ml. of the filtrate added to the isoniazid dilutions had no effect on the bacteriostatic activity of the compound, while larger amounts of filtrate progressively inactivated more isoniazid.

The "anti-isoniazid" substance appeared to inactivate isoniazid fairly rapidly. Dilutions of isoniazid to which had been added active filtrates of 607 and H37Rv and which were inoculated immediately with the test organism gave the same reduction of isoniazid inhibition of growth as dilutions of isoniazid containing the same active filtrate which were incubated at 37°C. for twenty-four to seventy-two hours before inoculation with the test organism.

Attempts to increase the yield of the "anti-isoniazid" substance by growing cultures of strains 607, H37Rv, H37RvR-isoniazid in the presence of a subbacteriostatic concentration of isoniazid (0.001 mg. per 100 ml.) failed. The concentration of isoniazid was increased in cultures of H37RvR-isoniazid to as much as 5.0 mg. per 100 ml. without obtaining an increase in the antagonistic activity of the filtrate.

The hydrogen ion concentration of the culture medium appeared to have little influence on the production of the "anti-isoniazid" substance. Filtrates of cultures of 607 grown at pH 9.0, 8.0, 7.0, 6.0, and 5.0 all reduced the bacteriostatic activity of isoniazid 64 fold with the exception of the culture grown at pH 9.0. The filtrate from this culture reduced the bacteriostatic activity only 16 fold. Moreover, the pH of active filtrates ranged from pH 5.5 (*E. coli*) to pH 8.8 (*M. phlei*).

TABLE 3

EFFECT OF AMOUNT OF FILTRATE FROM CULTURES OF MYCOBACTERIUM 607, H37Rv, AND H37Ra ON THE BACTERIOSTATIC ACTIVITY OF ISONIAZID FOR MYCOBACTERIUM 607

Amount of Filtrate Added to Isoniazid Dilutions	Source of Filtrate		
	H37Rv (52-Day Culture)	H37Ra (55-Day Culture)	607 (49-Day Culture)
	Fold Decrease in Isoniazid Activity	Fold Decrease in Isoniazid Activity	Fold Decrease in Isoniazid Activity
0.1	0	0	0
0.5	16–32	8	32
1.0	32–64	32	64
2.0	64–128	64–128	128

TABLE 4

THE EFFECT OF HEAT ON THE ANTI-ISONIAZID ACTIVITY OF FILTRATES FROM CULTURES OF 607, H37Rv, H37Ra, AND M. PHLEI

Temperature in Degrees Centigrade	Time in Minutes	Fold Decrease in Activity of Isoniazid			
		Strain of *Mycobacterium*			
		607	H37Rv	H37Ra	*M. Phlei*
56.0	5	32			
	10	16			
	30	4			
90.0	5	0	4–16	16	64
	15		8	2–4	
	30		2–16	0–2	32
98.0	30		4–8	0	32
104.0	5		16		
Control (room temperature)	0	64	32	32–64	64

The results obtained when *M. phlei* and H37Rv were employed as test organisms to determine the reduction in the bacteriostatic activity of isoniazid following the addition of active filtrates were similar to those obtained with the 607 strain.

Some indication of the chemical nature of the active substance was obtained from the results of the following four experiments.

Active filtrates from cultures of 607 and H37Rv were dialyzed against three changes of double distilled water for eighteen to twenty-four hours at refrigerator temperature. The contents of the dialysis sacs were then sterilized by sintered glass filtration, added to isoniazid dilutions, and inoculated with a suspension of 607. These materials antagonized the bacteriostatic activity of isoniazid to the same degree as the original nondialyzed filtrates.

Active filtrates from cultures of 607 and H37Rv were treated with an active preparation of the proteolytic enzyme, crystalline trypsin. One milligram of trypsin was added to 20.0 ml. amounts of filtrate, the pH of which was 8.0. The mixtures were sterilized by sintered glass filtration, allowed to stand overnight at 37°C., and were then added to the isoniazid dilutions and inoculated with 607. The "anti-isoniazid" activity of the filtrates was reduced 4 to 16 fold by this treatment.

The effect of heat on the activity of the "anti-isoniazid" substance was determined. The results are listed in table 4. The susceptibility to heat depended on the source of the filtrate. The "anti-isoniazid" substance was the most heat-susceptible in filtrates from cultures of 607; less susceptible when obtained from cultures of H37Ra, even more resistant from cultures of H37Rv, and most resistant to heat from cultures of *M. phlei*. Fairly consistent results were obtained from repeated determinations with all of the filtrates except those from cultures of H37Rv (table 4).

A filtrate of a 31-day-old culture of H37Rv was precipitated with saturated ammonium sulfate solution. The supernatant and the redissolved precipitate were dialyzed overnight, sterilized by sintered glass filtration, and added to the isoniazid concentrations. The "anti-isoniazid" substance was found solely in the precipitated fraction.

To determine whether the "anti-isoniazid" substance was present intracellularly as well as extracellularly, sterile aqueous cell extracts prepared from eight-day

cultures of 607 and from thirty-day cultures of H37Ra[3] were added to isoniazid dilutions and inoculated with 607. It was found that these extracts antagonized isoniazid inhibition even more than the filtrates of cultures of these organisms. One milliliter of the extract from cultures of 607 decreased isoniazid inhibition by 1,024 to 4,096 fold, 0.1 ml. decreased inhibition by 1,024 to 4,096 fold, 0.05 ml. by 256 to 2,048 fold, and 0.01 ml. by 2 to 8 fold. One milliliter of the extract of cultures of H37Ra decreased isoniazid inhibition by 128 to 2,048 fold, and 0.1 ml. decreased it by 256 fold.

The active substance in the extracts of these two organisms was found to be nondialyzable. The "anti-isoniazid" activity of the 607 extract was reduced 4 to 16 fold by trypsin and was almost completely inactivated by heat at 90°C. for five minutes. In addition, the "anti-isoniazid" substance appeared to be destroyed by a hydrogen ion concentration of pH 2.0 in both filtrates and extracts of cultures of 607.

From these findings the "anti-isoniazid" substance appeared to be a soluble intracellular substance which was either excreted into the culture medium during growth and multiplication of the organisms or was liberated into the medium following autolysis of the mycobacterial cells.

DISCUSSION

A substance has been found both in filtrates and in extracts of cultures of mycobacteria and in one strain of *E. coli* which is antagonistic to the inhibitory action of isoniazid on the growth of mycobacteria. The precise chemical nature of this "anti-isoniazid" substance is as yet undetermined. Nevertheless, it has been found to be nondialyzable, relatively heat-labile, partially inactivated by crystalline trypsin, destroyed by an acid pH (pH 2.0), and to be precipitated by ammonium sulfate. These findings suggest that the active substance may be a protein and possibly an enzyme. The nondialyzable property of the active substance and the fact that it was precipitated by ammonium sulfate would indicate that the activity was not due to compounds such as keto acids or pyridoxine (6–9). The possibility has not been excluded, however, that some other metabolite such as a porphyrin (10) might account for the "anti-isoniazid" effect.

Before too much significance is attached to the finding that the isoniazid-resistant strain of H37Rv produced less of the isoniazid-inactivating substance than did the isoniazid-susceptible strain of H37Rv, more strains of *M. tuberculosis* var. *hominis*, including strains made resistant to isoniazid both *in vitro* and *in vivo*, should be tested for their ability to produce the "anti-isoniazid" substance.

Apparently there are chemical differences in the "anti-isoniazid" substance, depending on the source of the inactivator. Although filtrates of cultures of *M. phlei* were just as active in reducing isoniazid inhibition as filtrates from cultures of 607 and H37Rv, the "anti-isoniazid" substance present in the *M. phlei* filtrate was heat-stable. Even more pronounced differences may be encountered between "anti-isoniazid" substances produced by other bacteria.

[3] The H37Ra extract was kindly supplied by Dr. Irving Millman.

SUMMARY

The demonstration that very large numbers of mycobacterial cells were less susceptible to the bacteriostatic action of isoniazid than smaller numbers led to the finding in filtrates of cultures of the mycobacterial strains H37Rv, H37Ra, 607, and *phlei* of a substance which inactivates isoniazid. The substance reduced the bacteriostatic action of isoniazid by 2 to 512 fold, depending upon the age of the culture from which the filtrate was taken. Aqueous cell extracts prepared by grinding cultures of 607 and H37Ra reduced the bacteriostatic activity of isoniazid 128 to 4,086 fold. An "anti-isoniazid" substance also was present in filtrates of cultures of *E. coli*.

Results are also presented which describe some of the conditions under which the "anti-isoniazid" substance is produced and which influence its activity.

A strain (H37Rv) made resistant *in vitro* to isoniazid produced very little of the "anti-isoniazid" substance.

SUMARIO

La Inactivación de la Isoniacida por Filtrados y Extractos de Micobacterias

El descubrimiento de que cantidades muy grandes de células micobacterianas eran menos susceptibles a la acción bacteriostática de la isoniacida que cantidades más pequeñas condujo al hallazgo, en filtrados de cultivos de las cepas micobacterianas H37Rv, H37Ra, 607 y *phlei*, de una substancia que inactiva la isoniacida. La substancia rebajó la acción bacteriostática de la isoniacida en 2 a 512 veces, conforme a la edad del cultivo del cual se tomó el filtrado. Los extractos celulares acuosos preparados triturando cultivos de 607 y H37Ra redujeron la actividad bacteriostática de la isoniacida 128–4086 veces. En los filtrados de los cultivos de *E. coli* también había una "anti-isoniacida."

Se presentan también resultados que describen algunas de las condiciones en las cuales se produce la substancia "anti-isoniacídica" y que afectan su actividad.

Una cepa (H37Rv) convertida *in vitro* en resistente a la isoniacida produjo muy poca substancia "anti-isoniacídica."

RESUME

L'inactivation de l'isoniazide par les filtrats et extraits de mycobactéries

La démonstration d'une moindre sensibilité à l'action bactériostatique de l'isoniazide des très grandes populations de cellules mycobactériennes par comparaison aux populations plus réduites a amené a découvrir dans les filtrats de cultures de souches mycobactériennes H37Rv, H37Ra, 607 et *phlei*, d'une substance réduisant de 2 à 512 fois l'action bactériostatique de l'isoniazide, selon l'âge de la culture à partir de laquelle le filtrat était obtenu. Des extraits aqueux de cellules obtenus par broyage de cultures de souches 607 et H37Ra réduisaient de 128 à 4 086 fois l'activité de l'isoniazide. Un "anti-isoniazide" était également présent dans les filtrats de cultures de *E. coli*.

L'auteur présente également les données démontrant certaines des conditions dans lesquelles la substance "anti-isoniazide" est produite et qui influencent son activité.

La production de la substance "anti-isoniazide" par une souche H37Rv rendue résistante à l'isoniazide *in vitro* était très faible.

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A TUBERCULOSIS OUTBREAK IN A MACACA MULATTA COLONY[1]

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INTRODUCTION

Recognition of tuberculosis as the outstanding single menace to the health of a monkey colony is well supported (1, 2). During a two-year period in the laboratories of the Yale University School of Medicine, a tuberculosis incidence of approximately 30 per cent of the monkey colony was reported (3). During a three-year period at the National Institute of Health, one-third of all monkeys received developed tuberculosis (4).

The present report presents the observations of a tuberculosis outbreak which occurred during February to May of 1953, in the *Macaca mulatta* (rhesus) colony of the Radiobiological Laboratory of the University of Texas and the United States Air Force at Austin, Texas. The magnitude of this outbreak warranted disposition of the entire colony.

HISTORY OF THE OUTBREAK

The incidence of tuberculosis prior to January, 1953, is not known, as vital colony data for this period were not accurately maintained. It is known, however, that tuberculous animals were discovered at autopsy prior to January, 1953, although the present writers were unaware of any instances in which tuberculosis was diagnosed ante mortem during this period.

In January, 1953, the concentration of tuberculin utilized for the intrapalpebral test was increased over that previously used as a measure to improve its diagnostic value. The new dilution was administered to one monkey which had exhibited a negative response to previous tuberculin tests but had displayed suspicious roentgenographic findings twenty-one days previously. This animal now exhibited a positive reaction to tuberculin. It was sacrificed in February for autopsy and displayed extensive miliary tuberculosis, confirmed through bacteriologic demonstration of the tubercle bacilli.

The maximum colony strength in February was 303 monkeys. Roentgenography and intrapalpebral tuberculin tests were given all monkeys. No positive ante-mortem diagnoses of tuberculosis were established on the basis of roentgenographic findings. A positive response to tuberculin was observed in 17 monkeys. One of these animals exhibited negative findings, and the remaining 16 were diagnosed as tuberculous at autopsy. Two monkeys which had exhibited negative tuberculin reactions were proved tuberculous at autopsy. The correlation of positive tuberculin reactions with confirmed tuberculosis at autopsy was approximately 84 per cent in this group.

The maximum colony strength in March was 288 monkeys. The intrapalpebral

[1] From the Radiobiological Laboratory of the University of Texas and the United States Air Force, Austin, Texas.

tuberculin test was administered to each animal. Positive responses to the tuberculin were observed in 12 monkeys. These animals were sacrificed, and tuberculosis was confirmed on each following post-mortem examination. The correlation of positive tuberculin reactions and positive autopsies in this test group was 100 per cent.

The maximum colony strength in April was 277 monkeys. The intrapalpebral tuberculin test was administered on April 7. Positive reactions to the tuberculin were observed in 24 animals. A second colony-wide tuberculin testing was accomplished on April 27. In this test, 11 positive reactors were observed. All positive reactors in the April 7 test were sacrificed prior to the April 27 test. Tuberculosis was confirmed in each of the 35 monkeys which exhibited positive tuberculin reactions. Tuberculosis was also diagnosed in one monkey which had exhibited a negative tuberculin reaction on the April 27 test. This monkey was sacrificed because of suspicious roentgenographic findings and associated illness. The correlation of positive tuberculin reactions and positive autopsies in the April tests was approximately 97 per cent.

The maximum colony strength in May was 223 animals. All positive reactors observed in the April tests were sacrificed prior to administering the intrapalpebral tuberculin test in May. Positive responses to tuberculin were observed in 21 monkeys in this test group. Autopsies on most of these monkeys were not accomplished in this laboratory and, therefore, post-mortem diagnoses are not accurately known; therefore, data on these are omitted from further consideration. With the exception of a group of 45 monkeys, all remaining animals in the colony were shipped elsewhere and eliminated. Since the experimental value of the group of 45 monkeys necessitated their perpetuation, they were moved to special isolation quarters and placed on therapy with streptomycylidene isonicotinyl hydrazine sulfate[2] (5). When moved into isolation, approximately 44 per cent of this group exhibited positive responses to the intrapalpebral tuberculin test. Apparently tuberculosis in these monkeys has been satisfactorily arrested through therapy, as only one death attributable to tuberculosis has occurred in this colony during the eighteen months since therapy was initiated. This monkey died ninety-one days following initiation of therapy.

CLINICAL DIAGNOSIS

Roentgenographic examination of each monkey in the colony has been an accepted procedure; however, it is of questionable value. In this outbreak, to the best of the writers' knowledge, no positive ante-mortem diagnoses of tuberculosis based on the findings of roentgenography have been confirmed.

The intrapalpebral tuberculin test is the principal index of diagnosis of tuberculosis in this laboratory. The test as employed here since January, 1953, consists of the intrapalpebral administration of 0.1 ml. of a 1:10 dilution of Koch's Old Tuberculin or 0.1 ml. of a 1:1.5 dilution of Standardized B.A.I. Mammalian Tuberculin, which provides an equivalent of 10 mg. of Koch's Old Tuberculin for each test. This test is similar to the tuberculin test recommended by Habel

[2] Supplied as Streptohydrazid® by Chas. Pfizer & Co., Inc.

(4), Kennard (3), and Schroeder (6) except that the tuberculin used in this laboratory is of greater concentration. The tuberculin test used in this laboratory prior to January, 1953, was almost identical to that recommended by Kennard (3), which consisted of the use of a 1:100 dilution of Koch's Old Tuberculin, so that each test was accomplished with 1 mg. of Old Tuberculin.

A positive test is indicated by a marked erythema and edema of the eyelids, occasionally accompanied by a small area of necrosis over the site of injection. No adverse systemic reactions have been observed. A positive reaction is usually visible in twenty-four hours and may increase in severity for forty-eight to seventy-two hours. If no reaction is observed within seventy-two hours, the test is considered negative. The extent of the reaction is generally immaterial if both erythema and edema are present, particularly during an outbreak of tuberculosis, and all monkeys thus showing sensitivity to tuberculin should be destroyed or otherwise promptly eliminated from any colony (6).

The clinical diagnosis of tuberculosis in monkeys by methods other than those described has not proved to be practical in this laboratory.

DIAGNOSIS AT AUTOPSY

Tuberculosis is as easy to recognize at autopsy in the monkey as in man (1). Diagnosis was established by gross observation at autopsy and stained smears of suspected material or stained tissue sections. The human strain of tuberculosis was identified in this outbreak.

TABLE 1

DISTRIBUTION OF TUBERCULOSIS IN 69 MACACA MULATTA MONKEYS

Site	Number of Animals
Lungs	64
Larynx	1
Pleura	2
Liver	29
Spleen	25
Lymph nodes	
Hilar	13
Mediastinal	5
Axillary	3
Mesenteric	4
Retroperitoneal	3
Inguinal	1
Gastrourinary tract	
Kidneys	6
Testes	2
Epididymis	1
Peritoneum	3
Heart (endocardium)	1
Pancreas	1
Bone (frontal)	1
Bone marrow	3
Skin	2
Muscle	1

Occasionally, small lesions in the lungs resulting from invasion by the lung mite, *Pneumonyssus foxi* (7), may be mistaken for tuberculous lesions. However, such lesions can usually be distinguished grossly from tubercles (8).

The usual type of tuberculosis found has been the miliary form involving the lungs, spleen, liver, and less frequently, the lymph nodes, kidneys, peritoneum, gastrointestinal tract, skin, and bone (table 1). The findings in the present study are similar to those reported by Kennard (9). Calcification was not observed, and little evidence was found to indicate that the disease was becoming naturally arrested.

Observations at autopsy revealed a very high correlation of the presence of gross evidence of tuberculosis with initial development of tuberculin sensitivity.

GENERAL COMMENTS

Macaca mulatta monkeys are apparently highly susceptible to tuberculosis as evidenced by the rapid spread of infection and rapid development of lesions in the monkeys of this colony.

The tuberculin test employed has proved to be an effective indication of the presence of active tuberculosis in the monkey. No evidence of development of tuberculin sensitivity as a result of repeated testing has been observed. The findings at autopsy were checked against the last tuberculin test performed. The correlation of tuberculin tests with autopsy findings in this study was approximately 94 per cent (table 2). This correlation approximates that reported by Habel (4) and exceeds that reported by Kennard and associates (1, 3) and Schroeder (6). The concentration of tuberculin used in this study was approximately twice that used by Habel and tenfold that reported by Kennard and Schroeder.

The correlation of negative tuberculin tests with the presence of tuberculosis was not fully determined, as these animals were not sacrificed for autopsy following any particular test period. Thus, the greatest source of inaccuracy in the tuberculin test is likely to be found in the false negative tests.

Due to a slight influx of new monkeys and the loss of many animals through research programs, it was virtually impossible to determine the true incidence of tuberculosis in the colony. On the basis of presented data, a tuberculosis incidence of approximately 22 per cent existed by the end of April. Evidence during the month of May indicated an incidence of approximately 30 per cent, which might well have increased if the colony had remained intact.

The procedure of tuberculin testing, with prompt removal and destruction of

TABLE 2

CORRELATION OF TUBERCULIN TESTS AND AUTOPSY FINDINGS

Number of tests administered	1,305
Maximum strength of colony	303
Tests positive/autopsy positive	64
Tests positive/autopsy negative	1
Tests negative/autopsy positive	3
Per cent correlation of tests and autopsies	94

positive tuberculin reactors in the colony, failed to alter the insidious course of the disease in this outbreak. However, this procedure has proved highly successful to date in the present colony. This tends to suggest that the length of contact with tuberculous monkeys is an important factor, and thus agrees with the conclusions of Habel (4).

No data are available on the length of survival following development of tuberculin sensitivity, as the majority of monkeys considered in this study were sacrificed within thirty days following such reactions.

SUMMARY

The data presented reflect the severity of an outbreak of tuberculosis in a *Macaca mulatta* monkey colony during the period of February to May, 1953.

The tuberculin test, consisting of the intrapalpebral administration of 10 mg. of Koch's Old Tuberculin, was the principal index of diagnosis of tuberculosis, and has proved to be highly effective. Observations at autopsy revealed a 94 per cent correlation between the tuberculin tests and the autopsy findings. No positive ante-mortem diagnoses of tuberculosis by roentgenography were confirmed in this study.

One group of 45 monkeys, 44 per cent of which exhibited positive responses to tuberculin, was moved to special isolation quarters and placed on therapy with streptomycylidene isonicotinyl hydrazine sulfate. One death attributable to tuberculosis has occurred in this group during the eighteen months since therapy was initiated. The disease apparently has not spread to other monkeys in the group. This has been indicated by routine quarterly tuberculin tests and by the absence of evidence of tuberculosis in 4 tuberculin-negative monkeys which died accidentally seven months following initiation of therapy.

SUMARIO

Brote de Tuberculosis en una Colonia de Macacos Mulatta

Los datos presentados reflejan la gravedad de un brote de tuberculosis en una colonia de monos de la especie *Macaca mulatta* durante el período de febrero a mayo de 1953.

La prueba de la tuberculina, consistiendo en la administración intrapalpebral de 10 mg. de la Tuberculina Antigua de Koch, fué el principal índice de diagnóstico para tuberculosis, mostrándose sumamente eficaz. Las observaciones hechas en la autopsia revelaron una correlación de 94 por ciento entre las reacciones a la tuberculina y los hallazgos autópsicos. En este estudio, no se confirmó ningún diagnóstico positivo de tuberculosis, hecho *ante mortem* con la roentgenografía.

Un grupo de 45 monos, 44 por ciento de los cuales manifestaron una respuesta positiva a la tuberculina, fué trasladado a un recinto especial de aislamiento y puesto a tratamiento con sulfato estreptomicilidénico-isonicotinílico de hidracina. Durante los dieciocho meses transcurridos desde que se iniciara la terapéutica, ha habido una muerte imputable a tuberculosis en este grupo. Al parecer, la enfermedad no se ha difundido a los otros monos del grupo, según indican la sistemática comprobación trimestral con tuberculina y la falta de signos de tuberculosis en 4 monos tuberculino-negativos que murieron fortuitamente a los siete meses de la iniciación de la terapéutica.

RESUME

Epidémie de tuberculose dans une colonie de Macaca mulatta

Les données présentées traduisent l'importance d'une épidémie de tuberculose dans une colonie de singes *Macaca mulatta* pendant la période Février–Mai 1953.

Le test à la tuberculine, consistant en injection intrapalpébrale de 10 mg de tuberculine de Koch(OT), constituait l'indice principal du diagnostic de tuberculose et il s'est montré extrêmement efficace. Les observations d'autopsie ont révélé une corrélation de 94% entre les tests à la tuberculine et les lésions constatées à l'autopsie. Dans cette étude, il n'y a eu aucun diagnostic radiologique ante-mortem positif confirmé pour la tuberculose.

Un groupe de 45 singes, dont 44% présentaient une réaction positive à la tuberculine, a été transféré dans les quartiers d'isolement spéciaux et soumis au traitement streptomycylidène-sulfate isonicotinyl d'hydrazine. Un décès imputable à la tuberculose s'est produit dans ce groupe, au cours des dix-huit mois qui se sont écoulés après l'instauration de la thérapie. La maladie ne semble pas s'être étendue aux autres singes de ce groupe, comme l'ont indiqué le test à la tuberculine trimestriel systématique et l'absence de signes de tuberculose chez 4 singes ayant une réaction négative à la tuberculine, qui sont morts accidentellement sept mois après le début du traitement.

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ADDITIONAL TUBERCULOUS ANTIBODIES DEMONSTRATED WITH ERYTHROCYTES SENSITIZED BY SUCCESSIVE ADSORPTIONS OF OLD TUBERCULIN AND FURTHER STUDIES ON THE ADSORPTIVE CAPACITIES OF TANNED CELLS[1]

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INTRODUCTION

Numerous investigations concerned with attempts to develop serologic procedures as aids in the diagnosis of tuberculosis have increased our awareness of the complexity of the antigen-antibody systems involved and have indicated the need for more specific test antigens (1).

Although the hemagglutination test of Middlebrook and Dubos (2) and the subsequent hemolytic modification of this procedure (3) have not proved successful as diagnostic techniques, they have provided means for studying the serologic response of experimentally infected animals and the sera of certain patients. For example, Dodd, Funkhouser, and Rheins (4) were able to demonstrate the existence of heat-stable antibodies in the sera from rabbits injected with several strains of mycobacteria and purified protein derivative (PPD). Hinson and associates (5) adapted erythrocytes sensitized to Old Tuberculin (OT) to an indirect Coombs' test and identified antibodies in human serum distinct from those measured by more direct methods.

Since it is generally conceded that intact erythrocytes adsorb carbohydrates more readily than proteins, Boyden (6) and Meynell (7) modified cells by treatment with tannic acid in order to remove protein antigens from PPD (Weybridge) and from extracts of bacillary masses.

The present paper describes the experimental results of testing tuberculous rabbit sera with erythrocyte suspensions which had been sensitized by successive adsorptions of the same OT and the effects of tanning on the adsorptive capacities of cells for certain antigens present in OT and PPD.

MATERIALS AND METHODS

Hemagglutination test and the hemolytic modification: The details of the techniques as employed in this laboratory have been reported elsewhere (8), but consisted essentially of treating 0.1 ml. of washed, packed sheep erythrocytes with 6.0 ml. of a 1:15 saline (buffered to pH 7.4) dilution of OT[2] for two hours in a 37°C. water bath. The cells were thrice washed with buffered saline and prepared as a 0.5 per cent suspension. Two drops of the sensitized cell suspension were added to each tube in a series containing an equal quantity of a doubling saline dilution of the serum to be tested. The cell serum mixtures were agitated, incubated in a 37°C. water bath for thirty minutes and, after light centrifugation, the results were read. The last serum dilution in which definite aggregates were observed after gentle agitation was recorded as the end point of the titration.

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[1] From the Department of Bacteriology, The Ohio State University, Columbus, Ohio.

[2] O.T. (4 X International Standard) was kindly supplied by Dr. H. D. Piersma, Lederle Laboratories Division, American Cyanamid Corporation, Pearl River, New York.

The hemolytic modification of this test was conducted precisely as the agglutination test described above, except that one drop of a 1:3 saline dilution of guinea pig complement[3] (twice adsorbed at refrigerator temperature for twenty minutes with one tenth volumes of packed, normal sheep cells) was added to each tube of the test. The last dilution of serum in which the cells were completely lysed after incubation was considered the end point of the lytic titration.

Whenever the procedures for the sensitization of cells were modified from the above during the experimentation, the details of the modifications will be described in the text.

Sera: Blood samples obtained from rabbits by cardiac puncture were permitted to clot at room temperature. After remaining overnight in the refrigerator, the removed sera were clarified by centrifugation. All sera were diluted 1:2 with buffered (pH 7.4) saline, heat inactivated at 56°C. for thirty minutes, and adsorbed twice with one-tenth volumes of packed sheep cells at 37°C. (each adsorption thirty minutes) to remove normally occurring antibodies prior to storage at minus 20°C. until needed.

Mycobacteria: Mycobacterium tuberculosis var. *bovis* (Ravenel), BCG, *M. tuberculosis* var. *hominis* (H37Rv), and *M. phlei* were cultivated on Petragnani medium and, in some instances, the avirulent organisms were cultivated on nutrient agar slants. The organisms were harvested in buffered saline and prepared as suspensions containing 2.0 mg. of organisms per ml. Homogeneity of the suspensions was accomplished by means of agitation with glass beads.

Young adult rabbits (approximately 2.0 kg. in weight) were injected intravenously with 2.0 mg. of the appropriate mycobacteria.

Control sera were obtained from all animals prior to the injection, and subsequent samples were obtained as indicated by the experiments, usually at weekly intervals.

PPD: The PPD used in this investigation was generously provided by Parke, Davis & Co., of Detroit, Michigan, and designated by them as Lots X-0266-A and 012585-A.

RESULTS

Sensitization of Erythrocytes by Repeated Adsorptions of Undiluted OT

In order to gain information regarding the multiplicity of antigens in OT, serial adsorptions were performed with sheep cells, suspensions of which were subsequently tested for their antigenicity.

> Four milliliters of undiluted OT were adsorbed with 0.1 ml. of packed sheep cells at 37°C. for two hours, the cells separated by centrifugation and stored as a suspension for subsequent testing, while the OT was re-adsorbed with fresh cells. This procedure was continued for twelve adsorptions. The various cell suspensions were tested by the hemagglutination and hemolytic techniques with rabbit sera prepared against several of the mycobacteria.

The complement employed for the lytic demonstrations was diluted 1:9 with saline, since preliminary experiments had shown that a 1:3 dilution contained naturally occurring antibody which reacted with cells sensitized with the concentrated OT. The higher dilution of complement had no effect on titration end points, as demonstrated by comparative tests. Successive adsorptions of OT rapidly removed the specific antigen against which the antibody in guinea pig serum was directed.

In addition to testing sera inactivated and adsorbed in the usual manner, aliquots of each sample were heated at 70°C. for ten minutes to determine if

[3] Dehydrated pooled guinea pig complement was purchased from the Texas Biological Laboratories and reconstituted as directed.

TABLE 1

THE EFFECT OF SUCCESSIVE ADSORPTIONS OF UNDILUTED OT ON THE SENSITIZATION OF SHEEP ERYTHROCYTES

Antisera Tested	Antibody Demonstration	Antigens												
		Cells Sensitized with 1:15 OT	Cells Sensitized by the Corresponding Adsorption of Undiluted OT											
			1	2	3	4	5	6	7	8	9	10	11	12
BCG	H.L. (1)	64*	512	256	128	32	16	8	1,024	512	1,024	256	128	256
	H.A. (2)	32	64	32	8	8	8	—	512	512	256	64	64	32
	H.L. (3)	16	32	16	32	16	8	—	16	16	8	8	4	8
	H.A. (4)	8	32	16	16	4	4	—	16	8	2	2	2	4
M. tuberculosis var. *bovis* (Ravenel)	H.L.	1,024	2,048	1,024	512	512	128	64	256	512	512	128	128	256
	H.A.	128	128	128	64	128	32	8	128	128	64	4	8	8
	H.L.	16	16	16	16	8	8	—	—	2	2	2	—	—
	H.A.	4	16	8	8	4	4	—	—	2	2	2	—	—
M. phlei	H.L.	1,024	16,384	4,096	2,048	512	256	64	1,024	1,024	1,024	256	128	256
	H.A.	512	256	256	512	256	128	32	512	256	256	128	32	32
	H.L.	32	64	32	16	32	16	2	16	8	16	8	4	8
	H.A.	32	32	32	32	8	4	—	16	4	2	2	4	2
M. tuberculosis var. *hominis* (H37Rv)	H.L.	16	256	32	16	4	—	—	—	—	—	—	—	—
	H.A.	8	8	4	4	2	—	—	2	2	—	—	—	—
	H.L.	2	—	—	—	—	—	—	—	—	—	—	—	—
	H.A.	2	4	—	—	—	—	—	—	—	—	—	—	—

* Titers expressed as reciprocals of dilutions; (1) hemolysin, (2) hemagglutinin, (3) heat stable hemolysin, (4) heat stable hemagglutinin.

the repeated adsorptions had removed the antigens responsible for the demonstration of heat-stable antibodies (4). The sera were chosen with concern only for their high titers against cells sensitized according to the usual procedure, and no effort was made to equilibrate antibody content by dilution.

As may be seen in table 1, there was generally a progressive decrease in serologic titers as cells from successive adsorptions 1 through 6 were tested; the hemolytic activity of the sera inactivated in the usual manner diminished most rapidly. As cells from the remaining adsorptions were tested, serologic activity was regained except in instances of serum prepared against H37Rv.

Repeated adsorptions of OT at room temperature and at 4°C. resulted in serologic patterns similar to, but not identical with, those just described.

Experiments with Tannic Acid

As mentioned previously, Boyden (6) demonstrated enhanced agglutinin titers with tuberculous sera if erythrocytes were treated with tannic acid prior to their sensitization with PPD (Weybridge). Since neither Boyden nor Meynell (7) referred to titrations with hemolysins, it seemed advisable to determine if such chemical treatment of cells would alter their capacity to adsorb the antigen or antigens present in OT or PPD which were responsible for the lytic demonstrations.

Accordingly, tannic acid (Mallinckrodt, Fluffy) was diluted to 1:10,000 with buffered saline (pH 7.4). One milliliter of this acid solution was mixed with 0.1 ml. of

TABLE 2

SEROLOGIC TITERS OF TUBERCULOUS SERA TESTED WITH NORMAL AND TANNED ERYTHROCYTES SENSITIZED WITH OT AND PPD

Cell Suspensions Tested	Rabbit Antisera							
	BCG		*M. phlei*		(*M. Tuberculosis* (Ravenel)		*M. Tuberculosis* (H37Rv)	
	H.L.	H.A.	H.L.	H.A.	H.L.	H.A.	H.L.	H.A.
Normal cells treated with 1:15 OT, two hours at 37°C.	512	64	1,024	256	256	128	128	32
Tanned cells treated with 1:15 OT, two hours at 37°C.	512	8	512	32	256	16	64	—
Normal cells treated with 0.45 mg. of PPD, thirty minutes at 37°C.	—	—	128	4	16	—	2	2
Normal cells treated with 0.45 mg. of PPD, one hour at 37°C.	—	—	128	4	16	—	2	2
Tanned cells treated with 0.45 mg. of PPD, thirty minutes at 37°C.	—	—	Not tested		16	—	2	2

packed sheep cells and incubated for ten minutes in a 37°C. water bath, after which the cells were washed three times with saline and treated with either 1:15 OT (6.0 ml.) or 1.0 ml. of PPD (Parke-Davis), containing 0.45 mg. of PPD per ml. The intervals of treatment are indicated below. Normal sheep cells were sensitized in a similar manner. The sera selected for testing were chosen without regard to the time lapse between the injection of antigen and the procurement of the blood sample.

The results recorded in table 2 indicate that tanning of cells apparently had no effect on their capacity to adsorb antigens from PPD, nor did sensitization for one hour result in serologic responses different from those observed after thirty minutes' sensitization. The hemolytic titers demonstrated with tanned cells sensitized with OT approximated those found with the "standard" procedure. The agglutinin titers, however, were either markedly decreased or absent. The serologic titers observed with PPD-treated cells bore no relationship to the results found with tanned or normal cells sensitized with OT.

The Testing of Sera Obtained at Intervals with Normal and Tanned Cells Treated with OT and with PPD-Sensitized Erythrocytes

In view of the above data and the serologic patterns reported as the result of titrating sera collected at intervals (4), the cell suspensions just described were tested with sera obtained by bleeding the animals weekly.

The results (table 3) not only verify the independence of the various hemolysins and agglutinins but the differences also emphasize the effect that time has on their appearance and titer.

On the basis of a comparison between titers shown with OT-sensitized and PPD-sensitized cells, arbitrary grouping of these sera was possible. Antisera

TABLE 3

THE TESTING OF SERA OBTAINED AT INTERVALS WITH NORMAL AND TANNED CELLS TREATED WITH OT AND WITH PPD-SENSITIZED ERYTHROCYTES

Antigen Injected	Rabbit	Weekly Serum Sample	Antigens					
			Hemagglutinin and Hemolysin Titers					
			OT-Treated Normal Cells		PPD-Treated Normal Cells		OT-Treated Tanned Cells	
			H.L.	H.A.	H.L.	H.A.	H.L.	H.A.
M. phlei	253	Normal	—	—	—	—	—	—
		1	128	32	4	4	128	8
		2	128	16	2	—	64	—
		3	64	16	4	2	64	2
		4	64	16	4	—	64	—
	255	Normal	—	—	—	—	—	—
		1	64	8	—	—	32	—
		2	128	8	—	—	32	—
		3	64	16	—	—	32	—
		4	64	8	—	—	32	—
BCG	A	Normal	—	—	—	—	—	—
		1	16	8	—	—	16	—
		2	256	32	—	—	64	—
		3	512	32	—	—	128	—
	D	Normal	—	—	—	—	—	—
		1	32	8	—	—	64	2
		2	256	32	4	2	64	4
		3	512	32	16	—	128	—
		4	256	2	16	—	128	—
	F	Normal	—	—	—	—	—	—
		1	32	8	—	—	32	2
		2	512	64	32	4	256	—
		3	1,024	64	32	4	256	2
M. tuberculosis var. *hominis* (H37Rv)	251	Normal	—	—	—	—	—	—
		1	32	4	—	—	16	—
		2	128	16	2	—	64	—
		3	128	32	2	2	64	—
		4	256	32	16	4	128	—
	252	Normal	—	—	—	—	—	—
		1	64	4	4	—	32	—
		2	128	16	4	2	64	—
		3	64	32	16	—	64	2
		4	128	32	16	—	64	—

TABLE 3—CONTINUED

Antigen Injected	Rabbit	Weekly Serum Sample	Antigens: Hemagglutinin and Hemolysin Titers — OT-Treated Normal Cells		PPD-Treated Normal Cells		OT-Treated Tanned Cells	
			H.L.	H.A.	H.L.	H.A.	H.L.	H.A.
M. tuberculosis var. *bovis* (Ravenel)	3	Normal						
		1	256	64	16	2	128	4
		2	512	64	128	8	512	8
		3	2,048	64	256	8	1,024	8
	4	Normal						
		1	32	8	4	—	32	—
		2	512	64	128	32	256	2
		3	2,048	256	256	256	1,024	32
	7	Normal						
		1	512	64	16	4	128	—
		2	1,024	64	256	8	1,024	4
		3	1,024	256	128	4	512	4
	8	Normal						
		1	128	32	16	—	64	—
		2	512	64	16	—	128	—
		3	1,096	64	256	8	2,048	4
	15	Normal						
		1	512	64	8	—	128	—
		2	512	128	64	8	128	—

to *M. phlei*, BCG, and H37Rv were similar in so far as no parallel existed between the lytic titers demonstrated with PPD-sensitized cells and those observed with the OT antigen. The lytic titers of the anti-Ravenel sera tested with PPD-sensitized cells more nearly paralleled the hemolytic titers found with OT-treated cells, both in time of appearance and in ascent.

All sera tested with OT-sensitized, tanned cells resulted in lytic titers approximately equal to those found with the standard OT antigen. In general, the agglutinin titers were again of a low level and agreed in trend with those found in similar tests in which PPD-treated erythrocytes were used as antigen.

DISCUSSION

The above data indicate that twelve successive adsorptions with normal sheep cells do not completely remove the polysaccharide antigens from Old Tuberculin. Moreover, the rate at which the various antigens are removed and the demonstration of others only on cells employed for the later adsorptions emphasizes the multiplicity and independence of the corresponding antibodies.

It is possible that competition may exist among the antigens present in the

antigenic mixture in OT for the same or similar sites on the erythrocyte. Conceivably, the carbohydrate antigens in OT differ both qualitatively and quantitatively and, therefore, are not adsorbed by sheep cells at the same rate. During successive adsorptions, the preferential removal of some antigens permits the late demonstration of those remaining. In addition, serologically nonreactive substances may be adsorbed to the erythrocytes and these may influence not only the total adsorptive capacity of the cell but also the rate at which the antigens may be removed from the mixture.

In any event, the usual techniques utilized for OT-sensitization of cells apparently prohibit the demonstration of many antibodies probably present in tuberculous sera.

The failure of this particular anti-H37Rv serum (*see* table 1) to exhibit patterns resembling those observed with the other antimycobacterial sera is attributed to antibody deficiency, since the sera from other animals tested more recently reacted typically.

The tanning of cells prior to sensitization with OT does not result in altered adsorption of antigens responsible for lytic demonstrations. Tanned cells, however, apparently are either unable to adsorb the entire complement of agglutinogens from OT or adsorb agglutinogens different from those removed with normal cells (*see* table 2).

The capacity of cells to adsorb antigens from small quantities of PPD (0.45 mg. per ml.) was not altered by previous tanning. It should be noted that no effort was made in this preliminary study to determine the quantity of PPD necessary for optimal sensitization. With regard to the results of Boyden and Meynell, it should be pointed out that these investigators examined the commercially prepared PPD (Weybridge), while the PPD employed in this study was provided by Parke, Davis & Co. Differences in the techniques of tanning and the quantities of PPD used to sensitize cells, as well as probable variation in the carbohydrate content of each product, may account for the apparent discrepancies in results. Moreover, no attempt was made to minimize or eliminate polysaccharide by dilution so that protein alone could be adsorbed by the tanned cells.

The impaired or altered capacity of tanned cells to remove agglutinogens from OT is reflected in all titrations. The erratic demonstrations of the corresponding agglutinins suggest that these antibodies differ from those revealed by the usual methods. A gross similarity between agglutinin patterns seen with tanned OT-sensitized cells and PPD-treated normal cells has already been mentioned.

SUMMARY

Successive adsorptions of Old Tuberculin (OT) with sheep cells permits the demonstration of antibodies in tuberculous sera which probably are directed against antigens not detected by the usual sensitizing procedures.

Tanned and normal sheep cells adsorb lytic antigens from OT equally well, but the adsorption of agglutinogens is different.

The tanning of cells does not affect the adsorption of antigens from small quantities of purified protein derivative (PPD).

SUMARIO

Nuevos Anticuerpos Tuberculosos Descubiertos con Eritrocitos Sensibilizados por Adsorciones Sucesivas de TA y Estudios Ulteriores sobre las Capacidades Adsorbentes de los Glóbulos Curtidos

Las adsorciones sucesivas de Tuberculina Antigua (TA) permiten descubrir anticuerpos en el suero tuberculoso que probablemente contrarrestan antígenos que no distinguen los habituales procedimientos sensibilizantes.

Los glóbulos curtidos y normales de oveja adsorben los antígenos líticos de la TA igualmente bien, pero es distinta la adsorción de aglutinógenos.

El curtimiento de los glóbulos no afecta la adsorción de antígenos de pequeñas cantidades del derivado proteínico purificado (DPP).

RESUME

Anticorps tuberculeux additionnels démontrés par les érythrocytes sensibilisés par adsorptions successives de vieille tuberculine (OT) et autres études sur la capacité d'adsorption des globules tannés

Des adsorptions successives de tuberculine de Koch sur globules de mouton permettent de démontrer dans le sérum tuberculeux la présence d'anticorps qui sont probablement dirigés contre des antigènes n'ayant pas été décelés par les procédés de sensibilisation usuels.

Les globules de moutons tannés et normaux adsorbent de la même manière les antigènes lytiques de OT, mais l'adsorption des agglutinogènes est différente.

Le tannage des globules n'affecte pas l'adsorption d'antigènes provenant de petites quantités de dérivés protéiques purifiés (PPD).

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A PHYSIOLOGIC AND CLINICAL STUDY OF FAILURES IN VITAMIN A METABOLISM IN TUBERCULOUS PATIENTS[1, 2]

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INTRODUCTION

Malnutrition has long been considered an important factor in susceptibility to tuberculosis, but the exact nutritional defects associated with decreased resistance have not been precisely defined. Search for a better understanding of the role of nutrition in resistance to tuberculosis became possible only when accurate tests were developed for the principal nutrients found in the body.

Within recent years certain specific nutritional deficiencies in tuberculous patients have been recognized. Although tests for the measurement of all important nutrients are not at hand, enough are available to demonstrate that tuberculous patients are worse off nutritionally than nontuberculous persons in several respects, and that the more extensive the disease, the larger the number of nutritional deficiencies and the greater their severity. Deficiency in vitamin A and vitamin C blood concentrations has been found in both minimal and moderately advanced tuberculosis. In far advanced tuberculosis not only are the concentrations of these vitamins lowered, but decreased values are found also for albumin, hemoglobin, calcium, and phosphorus (1).

Conceivably, low blood concentrations of specific nutrients found in tuberculous patients could be either a cause or a result of the disease. In an investigation of eleven hundred originally nontuberculous patients studied during a period of years, low blood concentrations of vitamins A and C correlated with the development of tuberculosis (2). In these cases nutritional deficiency clearly preceded the development of tuberculosis.

It was believed that further light could be thrown on this problem by abolishing nutritional deficiencies in a group of tuberculous patients by artificial supplementation of their diet with the substances in which their tissues and blood were deficient. The clinical progress of these patients could then be compared with that of patients in an otherwise similar group to whom no nutrient supplements were given. A long-range study with this objective has been under way for some years at The Charles Cook Hastings Home in Altadena, California (3).

PLAN OF STUDY

The investigation here reported was carried out on newly diagnosed, previously untreated, male patients who were suffering from moderately advanced tuberculosis when

[1] From The Charles Cook Hastings Home, Altadena, California.

[2] Presented in part before the Medical Session, as part of the session on *Medical Treatment of Tuberculosis*, at the Fiftieth Anniversary Meeting of the National Tuberculosis Association, Atlantic City, New Jersey, on May 20, 1954.

admitted to the hospital. The majority of the patients were veterans of World War II, twenty to forty-five years of age. All were kept on a diet which was liberal in quantity and varied from day to day to increase its attractiveness. The diet provided approximately 3,000 calories per day, or sufficient to ensure a weight gain, and included a daily allowance of 120 gm. of protein. It was estimated that, in general, the patients consumed 90 gm. of protein per day. Each patient drank at least six ounces of fresh orange juice daily. When normal weight was attained, the calorie value of the diet was lowered by reducing the intake of carbohydrate-rich foods.

The study was originally planned in 1943 and fully organized during the war years. The first patients in the investigation here detailed began their course of treatment in 1946. The original group was restricted to veterans with tuberculosis of minimal extent. Later, patients with moderately advanced disease were admitted. Patients were added to the series in a slow turnover from 1947 on. The accepted basic therapy of the period was strict bed rest, and this was practiced rigidly in all patients admitted to this study. Needless to say, chemotherapy was in its infancy at the time, and safe and effective regimens had not yet been established.

The procedure followed was entirely acceptable to the patients, all of whom were informed before entrance to the institution that antimicrobial drugs would not be used unless their subsequent clinical course indicated the advisability of this fortification of their treatment. No unusual dietary regimens were practiced by any of the patients prior to admission to the study. Change to the hospital diet was without incident. No patients in the investigation received collapse therapy or surgery for tuberculosis.

At the outset of the study, the patients were divided into three groups designated, respectively, *Group I*, *Group II*, and *Group III*. *Group I* was set up as a control group. *Groups II* and *III* were given specific dietary supplements.

Group I consisted of 37 patients. The subjects in this group consumed the same basic diet as the patients in the other groups and received in addition each day tablets, capsules, and intramuscular injections identical in appearance and manner of administration with those received by patients in the treated groups, but entirely of placebo character. *Group I* patients were given 180 ml. of a placebo preparation made for us by Sunkist Growers, Inc. and 180 ml. of fresh orange juice.

Group II contained 26 patients. The subjects in this group consumed the same diet as the patients in *Group I*, but received nutrient supplements in addition each day as capsules, tablets, or intramuscular injections. A total of 4 gm. of ascorbic acid was given, part in the diet and part as tablets, which were given in four divided doses. Ten 0.5 gm. tablets of brewers yeast (Atlantic Yeast No. 136), twelve 0.5 gm. tablets of raw defatted liver, and six 200 mg. tablets of medicitrin (vitamin P) were also given each day. The patients in this group received daily 360 ml. of orange juice fortified with crystalline ascorbic acid.

The 26 patients in *Group II* were subdivided further into three subgroups on the basis of intake of vitamin A and related food factors. One third of *Group II* received daily doses of 150,000 international units (I.U.) of synthetic vitamin A palmitate plus 5,000 international units of synthetic vitamin D in a water-dispersible form. In a few of these patients, whose plasma concentrations of vitamin A rose rapidly, the dosage of vitamin A was dropped to 100,000 I.U. per day. A second third of *Group II* received 100,000 I.U., in two intramuscular doses, of synthetic vitamin A palmitate in sesame oil per week. The third subgroup of *Group II* received 100,000 I.U., intramuscularly, of synthetic vitamin A alcohol in sesame oil per week.

Group III consisted of 15 patients. They consumed the same basic diet as the patients in *Groups I* and *II*, 360 ml. of orange juice daily and the same vitamin supplements as the patients in *Group II*, with the following exception. Vitamins A and D were given to *Group III* in a crude concentrate from cod-liver oil in the form of the nonsaponifiable residue (designated NSR). The patients in this group were started on 200,000 I.U. of vitamin A and approximately 7,500 I.U. of the natural vitamin D in the nonsaponifiable res-

idue. They were kept on this regimen until the plasma concentration of vitamin A exceeded 300 I.U. per 100 ml.; when that concentration was reached, the daily dose was reduced to 150,000 I.U. to keep the concentration at approximately 300 I.U. If the blood concentration still exceeded this amount, the daily dosage was further reduced by 50,000 I.U.

It will be noted that the essential difference between *Group II* and *Group III* was that the former received vitamin A as a synthetic product, while the latter received it in the form of a crude concentrate from cod-liver oil.

Routine examination of the patients in the study included the usual laboratory tests employed in tuberculosis hospitals, with tests for tubercle bacilli by examination of sputum and gastric contents. The gastric content bacteriologic studies were made with a series of three daily concentrated specimens, which were injected into the same three guinea pigs, so that each pig, in effect, received a pool of three concentrated specimens.

Routine study also included stereo chest roentgenographic examination every six weeks. Chest films were read by an independent observer who did not know the group placement of the patients. This observer was given the responsibility also of determining if and when a patient was to be removed from the research and sent elsewhere for other treatment. In general, as the study progressed into the period of well-established chemotherapy, this meant treatment with streptomycin-PAS and, later, isoniazid. The chemotherapy was supplemented, as appeared appropriate, by surgical measures.

Hemoglobin determinations, complete blood counts, and erythrocyte sedimentation tests were made at six-week intervals on all patients.

In addition to the routine laboratory and roentgenographic examinations listed, the following special tests were made in order to evaluate the nutritional status of each patient:

Plasma tests for vitamin A esters, vitamin A alcohol, carotene, and vitamin C at six-week intervals.

Serum protein, calcium, phosphorus, and phosphatase determinations at three-month intervals.

Biomicroscopic examination of the eyes to detect the presence and determine the severity of blood sludging and to detect the presence of Bitôt spots under the conjunctivae at the limbus near the palpebral line of closure was carried out.

OBSERVATIONS

Alterations in Nutrition Resulting from Vitamin Supplementation

It did not prove possible to abolish all nutritional deficiencies in any group. Data on nutritional deficiencies at the time of admission to the hospital and inclusion in the study are presented in tables 1 and 2.

It will be seen that the great majority of the 78 patients with moderately advanced tuberculosis exhibited lowered blood concentrations of vitamin C and connective tissue thickening of the gums at the time of entrance. It may be noted in passing that a high percentage of patients with minimal and far advanced tuberculosis in residence in the hospital, but who were not included in

TABLE 1

EVIDENCE OF VITAMIN C DEFICIENCY IN 78 CASES OF MODERATELY ADVANCED PULMONARY TUBERCULOSIS ON ADMISSION TO HOSPITAL

Lowered blood concentration	86 per cent
Connective tissue thickening of gums	82 per cent

TABLE 2

EVIDENCE OF VITAMIN A DEFICIENCY IN 78 CASES OF MODERATELY ADVANCED PULMONARY TUBERCULOSIS ON ADMISSION TO HOSPITAL

Night blindness	98 per cent
Bitôt spots	97 per cent
Blood carotene	Not lowered
Vitamin A ester	Not lowered
Lowered vitamin A alcohol	73 per cent

the study (which is limited to cases of moderately advanced disease) also showed signs of vitamin C deficiency. All of the 5 patients with far advanced disease showed each of these findings of vitamin C deficiency. In the 22 cases of minimal disease, the only completed observation was on the blood; in 23 per cent, lowered plasma vitamin C concentrations were found.

Similar deficiency was detected in the vitamin A concentration on entrance. The overwhelming majority of patients suffered from night blindness and exhibited Bitôt spots. Approximately three-quarters of the patients were found to have lowered values for vitamin A in the blood. Thirty persons had severely low plasma concentrations, below 26 γ or 85 I.U. per 100 ml. of plasma. Blood values for carotene and vitamin A ester were not lowered.

The good basic hospital diet, unfortified by vitamin supplementation, failed to improve plasma concentrations of the vitamins a great deal. Eleven of the 37 in *Group I* never developed normal plasma vitamin A concentrations. Thirty-two of the 37 never developed normal plasma ascorbic acid concentrations.

Vitamin supplementation, however, raised vitamin plasma concentrations significantly (table 3 and figure 1). Supplemental ascorbic acid given in doses sufficient to bring the total consumption up to 4 gm. a day to the 41 patients in *Groups II* and *III* raised the plasma content of vitamin C to more than 1 mg. per 100 ml. in two weeks in all 12 patients tested. Most of the 41 patients were not tested at the end of two weeks. The time of the second test was delayed as long as eight weeks in some cases. All of the patients had normal plasma concentrations on the second test. Tissue improvement in the gums, however, did not occur within the period of the study.

Plasma concentrations of vitamin A also improved in *Groups II* and *III*, but

TABLE 3

PERCENTAGE OF PATIENTS IN EACH TREATMENT GROUP WITH NORMAL PLASMA VITAMIN A ALCOHOL CONCENTRATIONS DURING THE FIRST 180 DAYS OF TREATMENT

Group	On Entrance	After 30 Days	After 60 Days	After 90 Days	After 120 Days	After 150 Days	After 180 Days
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Group I	27	37	42	38	51	57	54
Group II	23	57	73	66	63	67	68
Group III	25	96	87	88	100	91	100

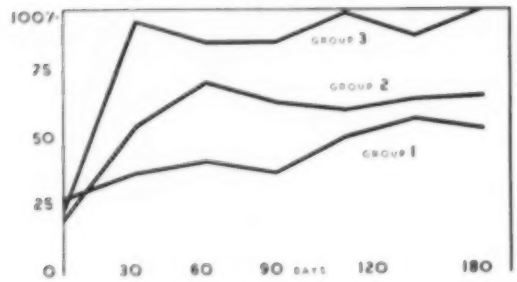

FIG. 1. Percentage of patients with plasma vitamin A alcohol concentration above 32γ per 100 ml. during first 180 days of treatment.

the improvement was not as rapid as with vitamin C (table 3). Vision, as indicated in the night blindness test, was improved in only 2 cases. Fleischer, Lubbers, and Schober (4) also failed to correct night blindness in tuberculous patients.

In the first third of *Group II* patients, who received synthetic vitamin A palmitate orally as a water-dispersed material (150,000 I.U. per day), a rise in the ester value in the plasma was evident in most instances, but little rise occurred in the blood concentration of vitamin A alcohol. In the other subgroups of *Group II*, when the synthetic vitamin A in either the ester (*Subgroup II*) or alcohol (*Subgroup III*) was injected intramuscularly, the dosage was reduced to 100,000 I.U. per week. This was done because of the higher rate of absorption with this method of administration, and the change in the plasma concentrations of both vitamin A forms was the least.

On the whole, the responses induced by vitamin A were slow and irregular. As noted, synthetic vitamin A supplements were given to 26 patients, while 15 received a crude concentrate of vitamin A from cod-liver oil. Plasma concentrations were changed most conspicuously and most rapidly by the crude concentrate of vitamin A. Ninety per cent of the patients who received the concentrate had normal concentrations of vitamin A within thirty days (table 3).

The phenomenon of blood sludging was not altered in any of the groups.

Clinical Results of Vitamin Supplementation

Clinically, differences in the course of tuberculosis were detected in the three groups of patients. The variation in the course of disease was most conspicuous in the *Group III* patients. The clinical course of *Group II* patients did not differ significantly from that of *Group I* (control) patients; nor were differences worthy of special note evident among the subgroups of *Group II*. For this reason the figures for clinical results in the *Group II* patients were combined for tabular purposes.

Clinical failures are recorded in table 4. It will be seen that failure of good clinical response occurred in 15 of 37 cases in *Group I* (control group without vitamin supplementation), and 10 of 26 cases in Group II (vitamin supple-

TABLE 4

CLINICAL FAILURES IN 78 CASES OF MODERATELY ADVANCED PULMONARY TUBERCULOSIS BY TREATMENT GROUPS

Treatment Group	Total Number of Cases	Evidence of Clinical Failure				
		Advancing Disease	Stationary Disease	Failure of Cavity Closure	Other*	Total
I	37	3	6	3	3	15
II	26	1	0	5	4	10
III	15	0	0	0	0	0

* Included were one case of perirectal abscess, one case of tuberculous laryngitis, 2 cases of tuberculous osteomyelitis, and 3 cases of tracheobronchial tuberculosis.

mentation with vitamin A in synthetic form). No failures occurred in the patients of *Group III*, who received vitamin A in the form of crude concentrate of cod-liver oil.

It may be noted in this connection that, in previous studies of cases of minimal tuberculosis, 2 of 10 cases in which vitamins were not supplemented were recorded as clinical failures, while among 10 patients whose diet was fortified with vitamin supplements, including vitamin A in the form of cod-liver oil concentrate, no clinical failures occurred.

Every effort was made to maintain the random placement of patients in one of the three study groups. It is believed that the findings, therefore, can be examined statistically.[3] The chi-square formula cannot be used, as one of the observed frequencies is zero. The binomial equation reveals that differences in results between *Groups I* and *III* are significant.

The probability of chance distribution leading to the result recorded is only 0.002, or two chances in a thousand.

The results observed in *Group I* and *Group II* are not significantly different from each other—by the same procedure, $p = 0.2$.

DISCUSSION

The results recorded above indicate that dietary vitamin supplementation tended to bring the originally low vitamin C and vitamin A blood concentrations of patients with moderately advanced tuberculosis to normal values. In contrast, a good hospital diet containing an excess of caloric requirements, an abundance of protein, and ordinary amounts of the vitamins failed to do so.

Special interest attaches to the effect of vitamin A supplementation. Vitamin

[3] The formula for studying the binomial distribution follows

$$P = \frac{(a+b)!\,(a+d)!\,(a+c)!\,(b+d)!}{a!\,b!\,c!\,d!\,(a+b+c+d)!}$$

Substituting, a equals 22, the number of patients in *Group I*, who did well; b equals 15, the number of clinical failures in *Group I*; c equals 15, the number of patients in *Group III* who did well; d equals 0, the number of clinical failures in *Group III*; ! equals factorial. $p = 0.002$.

A exists in two forms in the blood (5–7). The vitamin is stored in the body in the form of an ester. Vitamin A alcohol represents 90 to 95 per cent of the vitamin A in the systemic circulation. It is this form that is lowered in the tuberculous patient. He seems unable to release the alcohol from the ester combination.

Carotene conversion to vitamin A also appears to be blocked in tuberculous patients. Both carotene and vitamin A ester exist in the plasma of tuberculous patients in the same amounts as in normal persons, even when signs and symptoms of vitamin deficiency are developing or are already present (1, 8).

When the crude concentrate of vitamin A is administered, blood concentrations of vitamin A ester and vitamin A alcohol both rise, the former to a higher degree than the latter.

The difference in the effect of crude concentrate of cod-liver oil and synthetic vitamin A appears significant not only from the point of view of raising blood concentrations but also in the striking variation recorded in the proportion of clinical failures in patients to whom the two products were administered. It is noteworthy that no clinical failures occurred in the group of patients receiving the crude concentrate.

The clinical results suggest that a factor favorable to healing is present in the crude concentrate of vitamin A from cod-liver oil. Isomers of synthetic vitamin A are now available, and Wald and Hubbard (9) have reported that these have different biologic potency in the visual cycle. The results encourage further work, which is now under way, to find the active principle responsible for the effects noted with the crude concentrate.

SUMMARY

A study was organized to determine if nutritional deficiencies in tuberculous patients could be abolished, and if their removal would lead to improvement in clinical progress.

Seventy-eight patients with moderately advanced tuberculosis, all of them showing nutritional deficiencies, were placed on bed rest, with a good hospital diet, and subjected to nutritional study and treatment. None received chemotherapy, collapse therapy, or surgery for tuberculosis while in the study.

At the start of the investigation, most of the 78 patients were found to have abnormally low blood concentrations of vitamin A and vitamin C. Signs and symptoms of vitamin A deficiency were discovered in patients with normal carotene and vitamin A ester concentrations. This suggested failure of the tissues of these patients to convert carotene to vitamin A and to release vitamin A from vitamin A ester.

For purposes of the investigation, the 78 patients were divided into three groups: *Group I*, with no vitamin supplementation of the hospital diet; *Group II*, which received the same basic diet as *Group I*, plus supplementation with vitamin A in the form of the synthetic substance; and *Group III*, similar to *Group II*, which received vitamin A in the form of crude cod-liver oil concentrate instead of synthetic vitamin A.

The basic hospital diet, more than sufficient for calorie and protein requirements, with more than average amounts of vitamins A and C, did not raise the

original low plasma concentrations of tuberculous patients in these two vitamins significantly.

Therapy with vitamin A produced slow and irregular responses in vitamin A blood concentrations. The response was much better with the cod-liver oil concentrate than with synthetic vitamin A.

All patients in the group which received the cod-liver oil concentrate (*Group III*) had a good clinical course and were discharged as inactive cases of pulmonary tuberculosis. There were no clinical failures. In contrast, clinical failures occurred in both the control group and the group which received synthetic vitamin A.

SUMARIO

Estudio Fisiológico y Clínico de los Fracasos del Metabolismo de la Vitamina A en los Tuberculosos

Organizóse el estudio aquí presentado para determinar si podían abolirse las deficiencias nutritivas en los tuberculosos y si esa eliminación ayudaría a mejorar la evolución clínica.

A 78 enfermos que tenían tuberculosis moderadamente avanzada, mostrando todos ellos deficiencias nutritivas, se les impuso el descanso en cama, con una buena alimentación sanatorial, sometiéndolos a estudio y tratamiento de la nutrición. Mientras se les estudiaba, ninguno de ellos recibió quimioterapia, colapsoterapia o tratamiento quirúrgico para la tuberculosis.

Al iniciarse la investigación, se observó que la mayor parte de los 78 enfermos mostraban concentraciones sanguíneas anormalmente bajas de vitamina A y vitamina C. Descubriéronse signos y síntomas de avitaminosis A en enfermos que revelaban concentraciones normales de carotina y de éster de vitamina A, hecho este que sugiere incapacidad de los tejidos de esos enfermos para convertir la carotina en vitamina A y para desprender la vitamina A del éster de ésta.

Para los fines de la investigación, dividiéronse los 78 pacientes en tres grupos: testigo (*I*), sin complemento de la vitamina contenida en el régimen sanatorial; (*II*), con la misma alimentación básica que el *Grupo I*, pero, que recibió además complemento dietético de vitamina en forma de la substancia sintética; y un grupo final (*III*), semejante al *Grupo II*, que recibió vitamina A en forma de aceite de hígado de bacalao crudo concentrado en vez de la vitamina A sintética.

La alimentación sanatorial básica, más que suficiente para colmar los requisitos de calorías y proteína, y superando las proporciones medias de vitaminas A y C, no hizo subir mayor cosa las primitivas concentracioses bajas de esas dos vitaminas en el plasma de los tuberculosos.

La terapéutica con vitamina A produjo respuestas lentas e irregulares en las concentraciones sanguíneas de vitamina A. Las respuesta fué mucho mejor con el aceite concentrado de hígado de bacalao que con la vitamina A sintética.

Todos los enfermos del grupo (*III*), que recibió el aceite concentrado de hígado de bacalao, mostraron una buena evolución clínica, dándoseles de alta con tuberculosis inactiva. No hubo entre ellos fracasos clínicos. En contraposición, hubo fracasos clínicos tanto en el grupo testigo como en el que recibió vitamina A sintética.

RESUME

Etude physiologique et clinique des déficiences du métabolisme de la vitamine A chez le tuberculeux

L'étude qui est rapportée a été entreprise pour déterminer s'il était possible d'obvier aux carences nutritionnelles du tuberculeux et si leur abolition pouvait conduire à une amélioration des conditions cliniques.

Soixante-dix huit malades atteints de tuberculose au stade modérément avancé, présentant tous des carences nutritionnelles ont été mis au repos au lit et à une bonne alimentation sanatoriale, puis étudiés au cours d'un traitement nutritionnel. Pendant cette étude aucun d'eux n'a été soumis à la chimiothérapie, à la collapsothérapie ou à la chirurgie pour tuberculose.

Au début de l'investigation, on a observé chez la plupart de ces 78 malades des concentrations sanguines anormalement faibles en vitamine A et en vitamine C. Des manifestations de carence en vitamine A ont été observées chez des patients dont les concentrations en carotène et en esters de la vitamine A étaient normales; fait qui suggérait l'inaptitude des tissus à transformer le carotène en vitamine A ou à libérer la vitamine A de l'ester de la vitamine A.

Pour procéder à cette investigation, les 78 patients ont été divisés en trois groupes: un groupe de contrôle (I) sans adjonction de vitamines au régime alimentaire sanatorial; un groupe (II) ayant le même régime standard, mais avec adjonction de vitamine A sous la forme de substances synthétiques et un dernier groupe (III) qui recevait comme le groupe II l'alimentation standard plus la vitamine A sous la forme d'un extrait concentré d'huile de foie de morue au lieu de vitamine A synthétique.

L'alimentation sanatoriale de base, qui était plus que suffisante du point de vue des besoins en calories et en protéines, avec une quantité de vitamines A et C supérieure à la moyenne, n'accroissait pas de façon significative les concentrations initiales du plasma enregistrées pour ces deux vitamines.

Le traitement par la vitamine A provoquait une réponse lente et irrégulière des concentrations sanguines en vitamine A. La réponse était nettement meilleure avec le concentré d'huile de foie de morue qu'avec la vitamine A synthétique.

Chez tous les patients, faisant partie du groupe traité par le concentré d'huile de foie de morue (groupe III) l'évolution clinique a été favorable et ils ont quitté le sanatorium, leur tuberculose étant considérée stabilisée. Il n'y a eu aucun échec clinique. Au contraire, l'échec clinique s'est manifesté dans les groupes de contrôle et dans le groupe recevant la vitamine A de synthèse.

Acknowledgments

The writer is greatly indebted to the staff of The Charles Cook Hastings Home, who have made this work possible, especially to Dr. Carl H. Parker, who interpreted the chest roentgenograms without knowing the group placement of the patients; Mrs. Katharine Dunn, Miss Satsuki Uyeno, and Mrs. Ann Brand, of the laboratory; Miss Emma Hollett, the dietitian; Miss Marie Hanks and Miss Olga Anderson, Head Nurses. The writer is also indebted to Sunkist Growers, Inc., who have supplied all of the oranges used by the patients, the placebo orange juice, and the vitamin P materials; to Hoffmann-La Roche, Inc., who have supplied most of the vitamin A and ascorbic acid; to Endo Products, Inc., who supplied the water-dispersed vitamin A; to White Laboratories, Inc., who supplied the nonsaponifiable residue of cod-liver oil used on the first 15 patients in *Group III*. The study would not have been possible without the assistance of Dr. Esmond R. Long, who helped with the plan of the study, acted as consultant during its course, and aided with the final draft of this paper.

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Case Reports

BCG VACCINATION AND MEASLES[1]

D. F. CROSS

(Received for publication November 15, 1954)

In the following case report the instance of an unusual reaction to BCG vaccination is presented. It is fully realized that reactions of unexpected degree occasionally follow BCG vaccination. In the case under consideration, however, it is believed that the conditions under which the complications developed deserve particular attention and suggest a possible relationship between the severity of the reaction and the presence of an infection with measles.

CASE REPORT

The patient was an American Indian female three years and eight months old. On January 21, 1953, a chest film showed no evidence of disease and, on January 28, 1953, a tuberculin reaction (Mantoux, 0.01 mg.) was negative. Five months later, on July 4, 1953, a chest film revealed no evidence of disease and, on July 7, 1953, another tuberculin reaction (Mantoux, 0.01 mg.) was negative.

On July 8, 1953, BCG vaccination was performed by the scarification method as recommended by the Institute of Microbiology and Hygiene, University of Montreal. The vaccine used was Emulsion No. 619-11. The site of vaccination was the lumbar region just above the plane of the umbilicus.

On July 28, 1953, a rash characteristic of measles first appeared and was considered to be definitely fading by August 1, 1953.

On August 19, 1953, the patient again developed fever accompanied by a swelling over the sixth rib on the left. The axillary lymph nodes were enlarged bilaterally, and the spleen was palpable.

At the beginning of September, 1953, the swelling over the sixth rib on the left and the lymph node enlargement in the left axilla both became soft and ruptured, producing sinuses. On October 5, the sinuses and the swellings on the chest wall and in the axilla were still evident. At this time, there was no roentgenographic evidence of a lesion of the sixth rib. The Mantoux reaction (1:100 tuberculin) was strongly positive. On October 7, 1953, the child was admitted to the Miller Bay Indian Hospital. In the right axilla, there were two nontender masses, each approximately the size of a chestnut; no ulceration was present. In the left axilla a larger mass approximately the size of a small hen's egg was present, which felt like a number of enlarged lymph nodes matted together; the overlying skin was ulcerated. At the level of the sixth rib and close to the left nipple, a sinus and an ulcerated area approximately three quarters of an inch in diameter were present.

Few other abnormalities were found. The child was fairly well developed and nourished. Extensive post-impetiginous pigmentation over the trunk and some eczema around the ears were present. A chest roentgenogram showed no evidence of pulmonary or pleural disease. Bulging axillary folds were revealed on both sides, however, and, on the right side, there was a clearly defined rounded abnormal shadow, 2.5 cm. in diameter, which was interpreted as representing an axillary abscess. The gastric contents on three occasions were negative for *Mycobacterium tuberculosis* by culture. The erythrocyte sedimentation rate (Westergren) was 43 mm. in one hour. The scratches, three on each side, where the BCG had been given were still visible. The remainder of the investigation was negative.

[1] From the Miller Bay Indian Hospital, Prince Rupert, British Columbia.

Pus was aspirated from the abscess in the right axilla on October 10. The pus was sent to the Provincial Division of Laboratories, Vancouver, British Columbia. *Mycobacterium tuberculosis* was isolated by culture and the bacilli were injected into a guinea pig. On autopsy, the guinea pig showed no evidence of tuberculosis. It was inferred that the organisms were avirulent for the guinea pig and were of the BCG strain of *M. tuberculosis*. It was believed that the BCG organisms introduced by vaccination had traveled by the superficial lymphatics to the axillary lymph nodes and to the intercostal nodes on the left side, where they grew and produced abscesses.

The patient received no antimicrobial treatment. By the end of November, the lumbar scarifications had disappeared and the sinuses had closed. By January, 1954, the residual features were scars and small shotty axillary nodes. The patient was discharged on February 8, 1954.

COMMENT

It is safe to say that, in general, no difference exists between the pathologic behavior of the primary focus caused by BCG vaccination in the skin or intestines and that created by the common tuberculous infection in the lung or anywhere else. In both cases, extension takes place associated with specific immunobiologic changes. Shortly following BCG vaccination, various manifestations indicative of bacillary spread and the onset of tuberculo-allergy have been observed. These include regional lymphadenopathy, disseminated skin tuberculids, skin lesions reminiscent of lupus, generalized macular rash, lichen scrofulosorum, erythema nodosum, tuberculosis verrucosa cutis, iridocyclitis, phlyctenular conjunctivitis. Contrary to the ordinary tuberculosis, however, the BCG reaction is relatively soon overcome by the defense mechanism of the host, although there are rare exceptions to this rule. It is sufficient to refer to the case reported by J. Meyer and K. A. Jensen in the September, 1954, issue and the letter of E. F. James in the February, 1955, issue of The American Review of Tuberculosis and Pulmonary Diseases.

It has been shown that undesirable reactions may be due to an ill-chosen site of vaccination, faulty method and technique, increased potency of vaccine, and overdosage. Even after elimination of all possible errors, however, and standardization of procedure, a certain number of cases remain in which unknown or at least uncertain and unpredictable factors appear to play a role in the unusual response to BCG. Momentary immunobiologic states, individual constitutional peculiarities, environmental stress, concurrent or intercurrent nonspecific infections, to mention just a few, are some of the factors which have been mentioned in this connection.

At any rate, regional lymphadenopathy is the principal complication of BCG vaccination, and statistics give account of its occurrence in France, Holland, the Scandinavian countries, India, and elsewhere. Smaller figures have been brought forward in the English-speaking countries concerning the BCG vaccination limited to only minor professional or ethnic groups such as nurses, soldiers, Indians, Eskimos, et cetera. Nevertheless, there is general agreement that, the younger the child, the higher the incidence of lymphadenitis and that, generally speaking, suppurative processes are very rare indeed above the age limit of two to three years.

This leads to the case under discussion. The patient was of the unusual age of nearly four years and of good development and nutritional state for that age. In view of this, it seemed necessary to look for an explanation for the unusual reaction to BCG, and so attention was directed toward the sequence of events. The patient was given BCG vaccination on July 8 and developed objective evidence of measles on or about July 28. It seems reasonable to suppose that measles had been contracted approximately one week after vaccination, that is, during the very first phase of BCG spread. To pediatricians,

measles characteristically lowers resistance to infections in general and to tuberculosis in particular. In proportion to the severity of the disease, even the older child becomes vulnerable, like a crab without its shell. It is a time-honored clinical experience that measles tends to aggravate tuberculosis and the role of the measles in causing spread of tuberculosis was well appreciated in the pre-chemotherapeutic era. Today, however, when chemotherapy is being used extensively in tuberculosis, the adverse effect of the occasional intercurrent infection with measles usually remains subclinical and is consequently ignored. Yet it must be borne in mind that the temporary fading of the positive tuberculin test during measles and other ancient observations still hold true. Nor is there any reason to assume that the character of measles could have changed for the better in recent years. If that is so, one cannot get away from the conclusion that coincidental measles may have an untoward effect on the course of BCG vaccination. Although there is little in the literature to that effect, the hypothetical prerequisites for an unwanted spread of the BCG infection appear to be present.

Although these are reflections suggested by a single clinical observation and by theoretical speculation, nevertheless, they seem reasonable, and there is really no way at present of settling the issue with certainty. Apart from the academic interest, questions of practical importance may also arise from these considerations. Deferring BCG vaccination during a time of high prevalence of measles seems a sound principle. Moreover, with exposure to measles in a given case, it would seem to be a common sense measure to defer vaccination with BCG.

SUMMARY

A case has been presented to show extensive suppurative regional adenitis following BCG vaccination. The role of the attack of measles which occurred shortly after vaccination has been discussed. The admittedly speculative conclusion drawn is that measles may occasionally intensify the reaction to BCG vaccination. The coincidence of the two should possibly be avoided.

SUMARIO

La Vacunación BCG y el Sarampión

El caso presentado mostraba extensa adenitis supurativa regional consecutivamente a la vacunación con BCG. Se discute el papel del ataque de sarampión que ocurrió poco después de la vacunación. La conclusión reconocidamente hipotética que se saca es que el sarampión puede de vez en cuando intensificar la reacción a la vacunación con BCG. Debe evitarse, si es posible, la coincidencia de los dos.

RESUME

Vaccination par le BCG et rougeole

Présentation d'un cas montrant une adénopathie régionale suppurative étendue à la suite de vaccination par le BCG. Le rôle de la rougeole, qui est apparue aussitôt après la vaccination, est commenté. La conclusion, purement speculative, est que la rougeole peut parfois exagérer la réaction à la vaccination par le BCG. Il y a lieu d'éviter leur coincidence autant que possible.

TUBERCULOUS MENINGITIS DUE TO AN ORGANISM OF LOW VIRULENCE WITH SPONTANEOUS RECOVERY[1]

JAMES H. CULLEN

(Received for publication March 1, 1955)

The infrequency of spontaneous recovery from tuberculous meningitis is well known and is illustrated by the statement of Lincoln (1) that, in the twenty-five years of the existence of the Chest Clinic of the Children's Medical Service at Bellevue prior to the use of the antituberculous drugs, no child had recovered from tuberculous meningitis. However, sporadic cases of such recovery have occurred from time to time and, in 1931, Kramer and Stein (2), in a review of the literature, found 73 fairly well-proved cases of tuberculous meningitis with recovery. To be sure, six of the meningeal infections recurred subsequently and five of the recurrences ended fatally.

The finding, in pathologic specimens, of *Mycobacterium tuberculosis* of low virulence is uncommon. In the Communicable Disease Center, Chamblee, Georgia, a study was made by Patnode and associates (3) of 302 organisms culturally characteristic of human-type *Mycobacterium tuberculosis*. Progressive disease in guinea pigs was produced by 299 (99 per cent) of the organisms. One of the strains which failed to produce progressive disease was obtained from a BCG-induced lesion. Of the other two strains, one caused regional lymph node infection and the other produced no disease in guinea pigs. The writers suggested that the low virulence of these latter two organisms might have been due to prolonged subculture before guinea pig inoculation was done. In a bacteriologic and pathologic study of 123 cases of tuberculous meningitis by Macgregor and Green (4), of Edinburgh, all of the organisms were pathogenic for guinea pigs and 25.1 per cent were also pathogenic for rabbits, indicating they were a bovine strain. The writers did not mention finding any tubercle bacilli of low virulence.

In the case reported below, spontaneous recovery seemed to be occurring in the seven months prior to the discovery of the etiologic agent. The causative organism was found to be human-type *Mycobacterium tuberculosis* of low virulence for guinea pigs. Subsequent treatment with antituberculous drugs did not seem to change the clinical course of the disease.

CASE REPORT

The patient was a white male, 32 years of age, when first admitted to the Albany Veterans Administration Hospital on September 8, 1952. Three days prior to admission he had noticed the rapid onset of generalized aches, severe headaches, malaise, and temperature of 101°F. which persisted. When his symptoms did not respond to acetylsalicylic acid, he sought admission to the hospital.

The patient's past history revealed that he had had the usual childhood diseases and malaria in 1941, an appendectomy in 1941, and a herniorrhaphy in 1944. He had fractured the second, third, and fourth fingers of the right hand in 1941 and the right wrist in 1944. Ever since the patient entered the U.S. Marines in 1941, he had had recurrent gastric distress, headaches, and pains in his shoulders. He was discharged from the Marines in 1943 because of these complaints with a diagnosis of psychoneurosis.

Examination on admission to the hospital revealed an apprehensive, alert, tense male whose temperature was 104° F. and whose blood pressure was 120 systolic and 86 diastolic in mm. of mercury. His face was flushed; mild tenderness was present over the left pectoral

[1] From the Veterans Administration Hospital, Albany, New York.

TABLE 1

CEREBROSPINAL FLUID FINDINGS

Date	Cells	Total Protein	Sugar	Gold Curve	Remarks
9/9/52	191-78 per cent lymphocytes	69	82	C	Culture negative for *M. tuberculosis*
9/12/52	220-52 per cent lymphocytes	67	—	—	
9/25/52	10	87	—	—	
11/3/52	1	62	—	—	
2/4/53	0	63	—	C	
3/11/53	0	62	37	C	Cervical myelogram
3/17/53	7	66	86	—	Culture positive for *M. tuberculosis*
4/20/53	2	65	74	A B	Culture negative for *M. tuberculosis*
4/22/53	2	67	95	A B	Smear positive for acid-fast bacilli; culture negative for *M. tuberculosis*
4/23/53	—	—	—	—	Streptomycin, PAS, and isoniazid started
4/29/53	1	67	—	C	
6/15/53	2	74	86	C	
9/15/53	0	74	82	A B	
2/23/54	0	76	89	A B	
6/23/54	2	62	80	C	
10/18/54	1	62	92	—	

muscle; and moderate tenderness with voluntary splinting, but no spasm, was present in the right lower quadrant of the abdomen. There was mild nuchal rigidity, but otherwise there were no abnormal neurologic signs or reflexes. His optic fundi appeared normal.

Laboratory examination revealed a negative serologic test for syphilis, normal urine, a hemoglobin of 14.5 gm., with 4.9 million erythrocytes. The leukocyte count equalled 10,800, with 83 per cent neutrophils, 11 per cent lymphocytes, 5 per cent monocytes, and 1 per cent eosinophils. The chest roentgenogram showed a small discrete area of calcification in the left apex and another in the right mid-lung. These remained unchanged on all subsequent films. Serial cerebrospinal fluid findings are shown in table 1.

While in the hospital the patient's temperature gradually fell to normal during a four-day period and since then has remained normal. He did not receive any antimicrobial drugs before or during this hospitalization. His headaches and muscle aching were markedly reduced, but had not been completely relieved by the time of his discharge from the hospital on October 2, 1952, with a diagnosis of abortive, nonparalytic poliomyelitis. Following his discharge the patient continued to have occasional headaches and more and more pain in both shoulders. From time to time he also had some abdominal distress, but these symptoms were no different from those he had been having for the previous ten years.

On February 4, 1953, the patient was readmitted to the hospital for further examinations. At this time, roentgenograms of the skull were within normal limits except for a few pinpoint calcifications overlying the petrous ridges. There were no plaques of calcification noted about the meningeal areas. The pineal body was calcified and in its proper position. Roentgenograms of the shoulders showed small calcific densities adjacent to the tuberosities of both humeri suggestive of bilateral peritendinitis calcarea.

On March 11, 1953, a cervical myelogram was done and revealed no abnormalities. The patient was discharged from the hospital on March 27, 1953.

For three and one-half weeks after his discharge from the hospital, there was no change in the patient's condition. His migratory aches and pains continued but, he was "up and about"; however, he did not return to work. On April 20, 1953, he was readmitted to the hospital because the cerebrospinal fluid specimen obtained on March 17, 1953 (one week after the myelogram), showed *Mycobacterium tuberculosis* on culture.

At this time (April 30, 1953) the patient complained of intermittent frontal headache which radiated to the back of his head and was present mostly on the right side. There were no new physical findings, and nuchal rigidity was absent. The optic fundi again appeared normal. A cerebrospinal fluid specimen obtained on April 22, 1953, showed many acid-fast bacilli on smear which were strung out in a sort of linear fashion as though a pellicle had been smeared. However, no pellicle had been searched for or observed grossly. Culture of this cerebrospinal fluid specimen was sterile. Because of these two reports of finding acid-fast bacilli, one on culture and the other on smear, 1.0 gm. of streptomycin, 12 gm. of PAS, and 300 mg. of isoniazid daily were started on April 23, 1953. This chemotherapy was continued uninterruptedly except for the reduction of streptomycin to 1 gm. twice weekly on August 30, 1953, until December 22, 1954, when they were optionally discontinued. No intrathecal therapy was given.

Further studies during this hospital admission showed a 1-plus reaction to 0.0002 mg. of PPD, but no reaction to a 1:100 dilution of either histoplasmin or coccidioidin. An electroencephalogram on April 30, 1953, using monopolar and bipolar recording showed a few borderline abnormalities, probably an essentially normal record, with no evidence of focal or paroxysmal disorder. A roentgenogram of the spine and an intravenous pyelogram were normal. Repeated sputum, gastric content, and urine examinations were negative for tubercle bacilli on culture and guinea pig inoculation. There was no fever at all during this period, and the blood count always remained within normal range. The patient was discharged home on April 23, 1954, with no change in either his symptoms or general condition. He returned to work in October, 1954, in spite of continuing sporadic headaches, backaches, and abdominal pains.

The cerebrospinal fluid findings are shown in table 1. This includes all of the results prior to treatment and a representative selection of those obtained during treatment. Except for the initial tap on September 9, 1952, the cerebrospinal fluid pressures were always normal. The sugar was always normal except for the specimen obtained on March 11, 1953, when the cervical myelogram was done using Pantopaque®. This was not completely removed and, as recently as August, 1954, 1.5 ml. of Pantopaque® were removed when a lumbar puncture was done. It is not known how long an interval elapsed after the cerebrospinal fluid specimen of March 11, 1953, was obtained before the sugar determinations were done. It was because of this report of a low sugar that a repeat lumbar puncture was done on March 17, 1953. It was from this specimen that acid-fast organisms were cultured (two colonies) which were typical culturally and morphologically of *Mycobacterium tuberculosis*. The cerebrospinal fluid specimen of April 22, 1953, was the one with acid-fast bacilli on microscopy but sterile on culture as already described. All subsequent cerebrospinal fluid specimens were negative for tubercle bacilli by microscopy, culture, and guinea pig inoculation. Although other organisms, including *Cryptococcus neoformans*, were repeatedly searched for, none was found.

Several studies were done on the organism isolated from the cerebrospinal fluid specimen of March 17, 1953. A neutral red dye test, according to the method of Dubos and Middlebrook (5), gave a negative reaction suggestive of avirulence. On initial culture and repeated subculture, the organism never became chromogenic. Drug-susceptibility studies showed that this strain was susceptible to 3 γ of streptomycin, 10 γ of PAS, and 0.2 γ of isoniazid per ml. A guinea pig injected on April 22, 1953, with a subcultured specimen of the organism had only developed lesions of the spleen and local lymph nodes by the time it was killed and autopsied on June 22, 1953. Further studies of this organism

done a few months later by Edwin A. Broshe, bacteriologist of Sunmount Veterans Administration Hospital, showed that, on repeated guinea pig inoculations, only localized tuberculosis developed. No tuberculosis was produced in rabbits. He considered the organism to be *Mycobacterium tuberculosis*, human type, of low virulence. He also found that the strain was susceptible to streptomycin, PAS, and isoniazid.

COMMENT

The possibility that this case falls in the group described by Lincoln (1) as "serous meningitis" must be considered. She reported twelve cases in children, all of whom recovered. Sometimes an increased number of cells were in the cerebrospinal fluid, but the chemical findings were normal. Although she did not find any tubercle bacilli in the cerebrospinal fluid in her cases, she believed that the finding of a few organisms was not incompatible with serous tuberculous meningitis. The signs of meningitis were usually associated with a spread of tuberculosis locally or by the hematogenous route.

In the present case, the elevated protein, abnormal colloidal gold curve, and the absence of evidence of active spreading tuberculosis elsewhere would seem to remove the case from the category of serous meningitis. Moreover, the fact that the organism was recovered so long after the acute episode would not seem to fit serous meningitis.

Macgregor and Green (4) studied the cerebrospinal fluid in forty-five tuberculin reactors who only had symptoms of cerebral or meningeal irritation. Of these forty-five persons, only nine had active tuberculosis. In three instances tubercle bacilli were found on guinea pig inoculation, although these three children had no evidence of active tuberculosis. No meningitis was present in these patients or developed within the next two years, although paresis remained in one case. These same workers found in a post-mortem bacteriologic study of cerebrospinal fluid that, in twenty cases without gross or microscopic evidence of exudative meningitis, tubercle bacilli could be recovered in ten instances. In seven of these ten cases, tuberculous foci were found in the brain. They believed "that tuberculosis of the central nervous system, in common with other forms of the disease, may be arrested and undergo clinical cure, even when the cerebrospinal fluid has been infected, provided the lesions localized and that the diffuse exudative reaction does not take place".

Again it is hard to fit the present case into the type described above because the tubercle bacilli were found six and seven months after the acute episode, at a time when the cerebrospinal fluid was still abnormal.

As the first appearance of the unusual microorganisms in the cerebrospinal fluid was detected one week after the intrathecal instillation of Pantopaque®, it is conceivable that they were introduced with this radiopaque material. This question cannot be conclusively answered, especially as only one cerebrospinal fluid specimen had been examined for tubercle bacilli prior to that time and the positive culture was obtained six days after the introduction of the drug. It seems unlikely that this occurred, as one would have expected more of a meningeal reaction after the myelogram than did occur (as the organism did consistently produce local disease in guinea pigs) and, too, it seems more probable that the initial infection of the meninges occurred in September, 1952.

In any event, from the observations and studies during the course of the illness in this patient, it is believed that there is justification for considering that the case represents an instance of tuberculous meningitis in which spontaneous recovery was taking place and that the disease was due to a human-type *Mycobacterium tuberculosis* of low virulence. Since the patient did not receive any antimicrobial drugs prior to the isolation of the organism the possibility that nonspecific antimicrobial therapy altered the course of the

disease is eliminated. Antituberculous therapy did not seem to alter either the clinical course of the disease significantly or the cerebrospinal fluid findings. It is impossible to state whether the persistence of the elevated cerebrospinal fluid protein and the abnormal colloidal gold curve were due to the disease or to the retained Pantopaque® in the subarachnoid space. It is unfortunate that only one positive culture was obtained so that the characteristics of the organism could not be confirmed on another specimen. At least the finding of acid-fast bacilli on microscopy in addition to the isolation of a *Mycobacterium* by culture corroborates their active presence in the cerebrospinal fluid. Further speculation about the course of the disease and the organism recovered seems unjustified from the facts available. Nevertheless, this case raises the question whether some of the reported spontaneous recoveries from tuberculous meningitis in the past might not have been due to a similar organism of low virulence for guinea pigs and man.

SUMMARY

A case of tuberculous meningitis is reported which was caused by human type *Mycobacterium tuberculosis* of low virulence for the guinea pig and in which spontaneous recovery was taking place at the time that the presence of infection was detected.

SUMARIO

Meningitis Tuberculosa Debida a Microbio de Poca Virulencia, con Curacion Espontánea

Comunícase un caso de meningitis tuberculosa ocasionada por un *Mycobacterium tuberculosis* de tipo humano, dotado de poca virulencia para el cobayo, y en el cual ya iba teniendo lugar la curación espontánea al descubrirse la presencia de infección.

RESUME

Méningite tuberculeuse due à un germe de faible virulence et guérison spontanée

Le cas rapporté concerne une méningite tuberculeuse causée par un *Mycobacterium tuberculosis*, type humain, possédant une faible virulence pour le cobaye. La guérison s'est manifestée spontanément au moment où l'infection fut décelée.

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(4) Macgregor, A. R., and Green, C. A.: Tuberculosis of the central nervous system with special reference to tuberculous meningitis, J. Path & Bact., 1937, *45*, 613.

(5) Dubos, R. J., and Middlebrook, G.: Cytochemical reaction of virulent tubercle bacilli, Am. Rev. Tubere., 1948, *58*, 698.

Notes

THE IN VITRO ACTION OF CYCLOSERINE ON M. TUBERCULOSIS

Cycloserine is a new antimicrobial drug which in a preliminary trial[1] has shown some promise in the treatment of tuberculosis. The present report has to do with the effectiveness of cycloserine against *Mycobacterium tuberculosis in vitro*.

The following strains were used in this study:

H37Rv: This virulent human strain was received from William Steenken, Jr., of the Trudeau Laboratories. It is susceptible to isoniazid, streptomycin, and para-aminosalicylic acid (PAS).

H37Rv—isoniazid-resistant: This is a variant of the above parent strain and is resistant to more than 15γ of isoniazid per ml. of medium. Its resistance was developed by serial transfer in the laboratory. It is catalase-negative.

H37Rv—streptomycin-resistant: This strain is resistant to more than 50γ of streptomycin per ml. of medium and was developed by serial transfer in the laboratory.

H37Rv—PAS-resistant: This strain is resistant to more than 40γ of PAS per ml. of medium and was received from William Steenken, Jr., of the Trudeau Laboratories.

R1Rv: This strain was received from Trudeau Laboratories and is susceptible to isoniazid, PAS, and streptomycin.

BCG: This strain was received from Sol Rosenthal, of the Tice Laboratories, Chicago.

All studies were performed using Dubos liquid Tween®-albumin medium[2]. Tubes containing 9.5 ml. of medium were inoculated with one-half million to one million viable units in 0.5 ml. of Dubos liquid medium, and the drug was added to each tube in 1 ml. of saline. Control tubes received 1 ml. of saline. A set of five tubes was used for each concentration of drug tested, and growth in all tubes was measured daily nephelometrically.

In the Warburg studies, 2.5 ml. of Dubos Tween®-albumin liquid medium suspending R1Rv (2,250 nephelometric units per ml.)[3] were used in each vessel and 60γ of cycloserine in 0.5 ml. of saline were tilted in from the side arm of the vessel. One-half milliliter of saline was tilted into each control vessel.

To measure the effect of cycloserine on the normal growth curve of tubercle bacilli, the drug was added to H37Rv growing in 20 ml. of Dubos liquid Tween® albumin medium in a special flask designed to facilitate nephelometric readings and at the same time to support sustained logarithmic growth.

When cycloserine was added to the medium at the time of inoculation and the growth of the organisms was followed nephelometrically, growth curves shown in figure 1 were obtained. By arbitrarily selecting a nephelometric value at which growth is in the arithmetic phase and measuring the distance between the curve for the control tubes and those of the treated tubes, one can express the effectiveness of the antimicrobial in terms of "days' delay in growth." For example, in figure 1, at 80 nephelometric units, 2.5 γ of cycloserine per ml. delayed growth 0.4 days and 5.0 γ delayed growth 2.5 days. The value 0.4 days is not a significant one and, therefore, the drug concentration of 5.0 γ of cycloserine per ml. of medium was considered the minimal inhibiting dose in this experiment. A concentration of 10 γ of cycloserine per ml. produced permanent inhibition in terms of the duration of the experiment (10 days).

[1] Fourteenth Conference on Chemotherapy, Veterans Administration, Army, Navy, Atlanta, Georgia, February, 1955.

[2] Dubos, R. J., and Davis, B. D.: J. Exper. Med., 1946, *83*, 409.

[3] Fisher, M. W., and Kirchheimer, W. F.: Am. Rev. Tubere., 1952, *66*, 758.

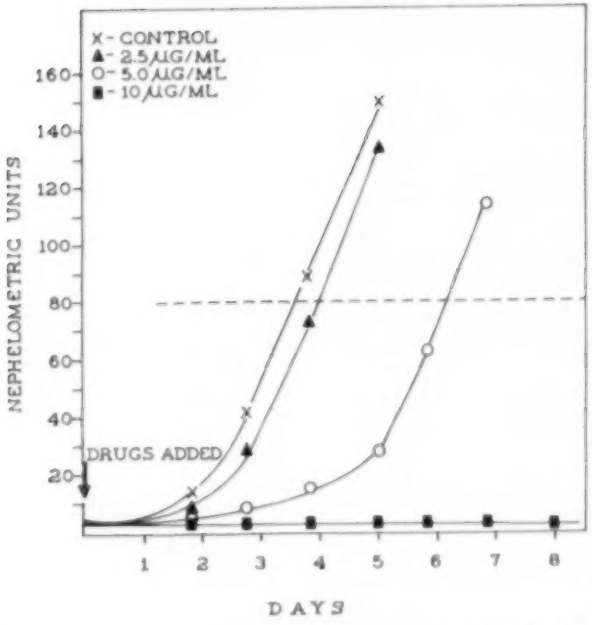

FIG. 1. Effect of increasing concentrations of cycloserine on growth of H37Rv, added at the time of inoculation.

TABLE 1

GROWTH INHIBITION OF DIFFERENT STRAINS OF M. TUBERCULOSIS BY INCREASING CONCENTRATIONS OF CYCLOSERINE ADDED TO LIQUID MEDIUM AT THE TIME OF INOCULATION

Organisms	"Days Delay in Growth" with Cycloserine			
	2.5 γ per Ml.	5.0 γ per Ml.	10 γ per Ml.	20 γ per Ml.
H37Rv	0	2.5	>10.0	>10
H37Rv, PAS-resistant	0	4.5	>10.0	>10
H37Rv, isoniazid-resistant	0	1.9	>10.0	>10
H37Rv, streptomycin-resistant	0	0.8	2.9	>10
RIRv	0	0.0	4.0	>10
BCG	0	0.0	3.0	>10

The effects of cycloserine on the different strains of mycobacteria described is summarized in table 1 in terms of "days' delay in growth."

It is apparent from table 1 that no pronounced difference exists between any of the strains tested. Streptomycin-resistant bacilli and the avirulent strains were slightly less susceptible to cycloserine than was H37Rv, and the PAS-resistant strain was slightly more susceptible than was the parent strain.

The effect of adding cycloserine to an actively growing culture of H37Rv in Dubos

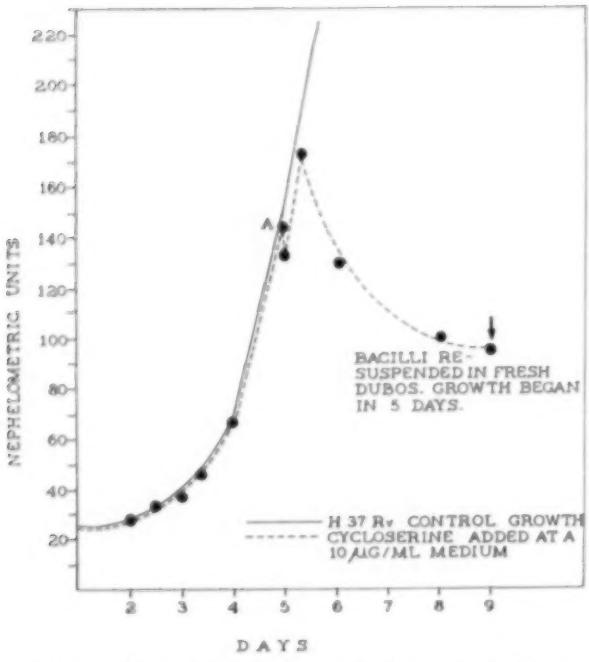

FIG. 2. Effect of cycloserine on actively growing H37Rv

liquid medium is shown in figure 2. Cycloserine (10 γ per ml.) was added to the flask on the fifth day, when the culture had grown to 144 nephelometric units. Addition of the drug in 1 ml. of saline caused dilution of the suspension of organisms and reduced the nephelometer reading to 131 units. The organisms continued to multiply, and nine hours after the drug had been added the reading was 174 units. At approximately this time growth ceased and the density of the culture began to decrease. On the ninth day, when the nephelometric reading had fallen to 96 units, a sample of the culture was stained by the Ziehl-Neelsen method. The stained smear revealed only approximately 10 per cent of acid-fast rods. The remaining material consisted of nonacid-fast bacilli, broken and disintegrated in appearance, plus considerable amorphous nonacid-fast material. The remainder of the cells were separated by centrifugation and reinoculated into five tubes containing 10 ml. of fresh Dubos liquid medium. These showed no growth for 5 days and then typical acid-fast bacilli grew out.

In figure 3 the effect of combining a subinhibiting dose of PAS with a subinhibiting dose of cycloserine is demonstrated. Some additive effect of the two drugs could be observed but there was no synergistic effect at the concentration of 1 γ of PAS with 3 γ of cycloserine per ml. of medium.

In figure 4 the effect of adding cycloserine to an actively growing culture of H37Rv is compared with the effect of adding cycloserine to a similar culture to which streptomycin had been added eleven hours earlier. At the time marked *A*, streptomycin (10 γ per ml. medium) was added to flask 1. This slowed but did not arrest the growth of the

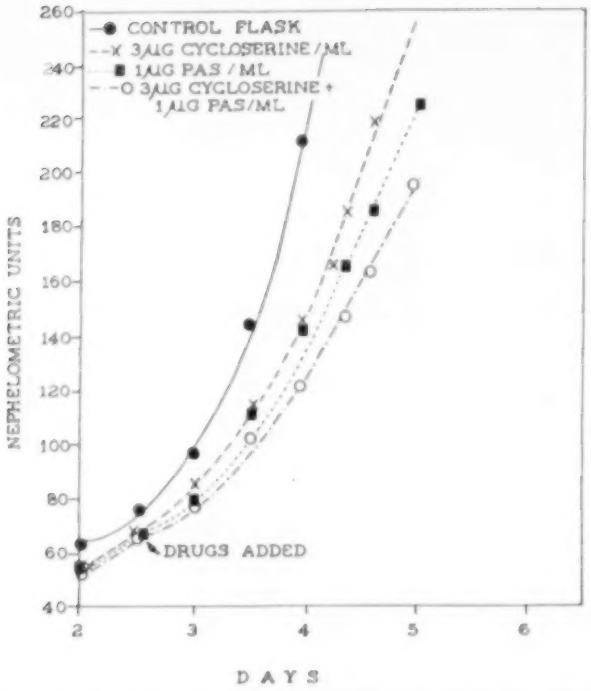

FIG. 3. Effect of a combination of cycloserine and PAS on growing H37Rv

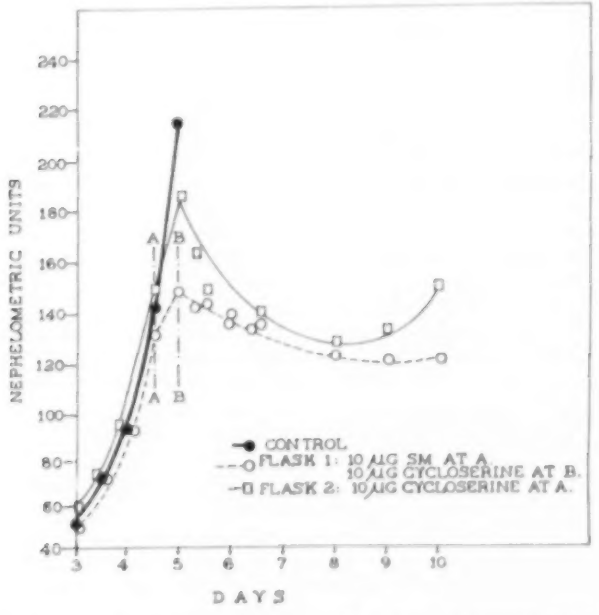

FIG. 4. Comparison of the effect of cycloserine on actively growing H37Rv, with and without previous addition of streptomycin.

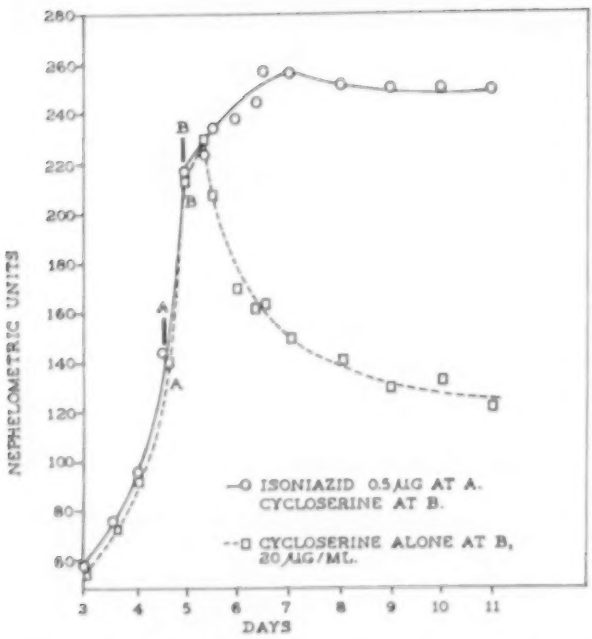

FIG. 5. Comparison of the effect of cycloserine on actively growing H37Rv, with and without previous addition of isoniazid.

bacilli. Eleven hours later at time marked *B*, cycloserine (10 γ per ml.) was added to flask 1 and growth ceased immediately and the bacterial density decreased slowly. This type of curve is also seen with streptomycin alone. Smears of the cultures on the tenth day showed that the bacilli in this flask were completely disintegrated. A large number of spherical poorly staining bodies were present, but no typical acid-fast bacilli were seen. Flask 2 received cycloserine (10 γ per ml.) at time marked *A*, and growth continued for eleven hours and then fell sharply and finally paralleled the bacterial density in flask 1 until the seventh day, at which time the bacterial density increased again. By the tenth day a Ziehl-Neelsen stain showed a mixture of typical acid-fast bacilli and nonacid-fast, poorly staining ghost cells and cellular debris.

In figure 5 the effect of adding isoniazid followed by cycloserine eleven hours later is shown and the result is compared with adding cycloserine alone. Adding the isoniazid at time *A* did not slow the bacterial growth in the first eleven hours and the growth curve was superimposed on the control curve. When cycloserine was added to the isoniazid-treated bacilli at time *B*, growth continued until the bacterial density reached 260 units and then formed a plateau. This is the type of curve which would be anticipated with isoniazid alone[4]. The addition of cycloserine alone at point *B*, however, resulted in a short period of bacterial growth followed by a pronounced drop in the bacterial density as the cells disintegrated. On the eleventh day a smear from each flask was stained by the Ziehl-Neelsen technique. The culture treated with cycloserine alone showed a very occasional acid-fast bacillus and, for the most part, the material was nonacid-fast, amorphous debris.

[4] BARCLAY, W. R., *et al.*: Am. Rev. Tubere., 1953, *67*, 490.

The culture which had been treated with isoniazid and then cycloserine consisted of non-acid-fast rods with dark beading. There did not appear to be any disintegration of the cells and the smear closely resembled the appearance of organisms treated with isoniazid alone[5].

The metabolism of bacilli treated with cycloserine was examined by the Warburg technique and by the addition of tetrazolium. Tetrazolium reduction in the cultures used required at least twelve hours for color development in the controls. Therefore, the tetrazolium reduction test did not indicate what occurred in the first few hours after the addition of cycloserine. When tetrazolium was added to the medium at the time the drug was added, reduction to formazan was of a slightly less degree than in the control tubes. When tetrazolium was added twenty-four hours after the cycloserine, there was no reduction to formazan. When cycloserine (20 γ per ml.) was tilted into cultures of R1Rv in a Warburg apparatus, no effect was shown on the oxygen consumption for six hours after the addition of the drug. Apparently cycloserine does not immediately arrest the metabolism of tubercle bacilli.

* * *

From the above observations, it appears that cycloserine is an effective bacteriostatic drug against *M. tuberculosis in vitro*. The inhibiting dose, 5 to 10 γ per ml., is effective against virulent and avirulent strains and against bacilli that are resistant to streptomycin, PAS, or isoniazid. Subinhibiting doses of cycloserine are not synergistic with PAS to produce full bacterial inhibition. There is some indication (figure 4), however, that a combination of streptomycin and cycloserine will arrest growth for a longer period of time than will cycloserine alone. In this regard the effect of drug combinations on delaying the emergence of cycloserine-resistant bacilli will require careful study.

Although cultures exposed to cycloserine undergo bacterial disintegration after an initial brief period, the combination of isoniazid with cycloserine seems to prevent this disintegration. This suggests that disintegration of the bacilli is not a consequence of mere physical contact between drug and cells, but results from the bacilli either metabolizing the cycloserine or being forced into an abnormal metabolic pathway by the presence of the cycloserine and then subsequently disintegrating.

In summary: Cycloserine is bacteriostatic against *M. tuberculosis in vitro*. It inhibits the growth of virulent bacilli, avirulent bacilli, and organisms resistant to streptomycin, PAS, and isoniazid. It causes gradual disintegration of tubercle bacilli in growing cultures.

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May 9, 1955

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STREPTOMYCIN AND PARA-AMINOSALICYLIC ACID IN PULMONARY TUBERCULOSIS

I. Results *During* Treatment: 528 Patients

In late 1951, four New York State tuberculosis hospitals[1] began a cooperative study to evaluate the comparative efficacy of two streptomycin regimens in proved pulmonary tuberculosis. After being selected for chemotherapy, in staff conference, patients were assigned to the two regimens by alternation. Streptomycin was given in a single injection of 1 gm. daily to one group and thrice weekly to the other. All patients also received para-aminosalicylic acid (PAS) orally, 8 to 12 gm. in divided daily doses. All were on modified bed rest. The duration of treatment was decided individually for each patient on the basis of the "response" of his disease, unless untoward drug reaction necessitated premature termination. Each was to have received at least four months of chemotherapy, plus additional two-month periods as deemed indicated. Due mainly to early toxicity, chemotherapy was discontinued in less than four months in 108 persons, approximately 20 per cent of the original series of 528. Comparability of the two groups was not altered by these early withdrawals. The mean length of chemotherapy was slightly more than ten months for each group. Among the 420 patients who underwent chemotherapy for four or more months, the average length of treatment was approximately twelve months.

The present preliminary report concerns results *during* treatment. A later one will present the long-term results *following* treatment.

The original study group consisted of 528 patients, with a median age of thirty-four years; 55 per cent were males. Classified by 1950 *Diagnostic Standards of the National Tuberculosis Association*, 18 per cent of the group had minimal involvement, 51 per cent moderately advanced, and 31 per cent far advanced; 55 per cent evidenced cavitary disease. Fourteen per cent of the patients also had extrapulmonary tuberculosis, and 9 per cent had significant nontuberculous complications. In 11 per cent, previous thoracic collapse or excisional therapy had been performed. Chemotherapy of four months or longer was completed in 420 patients.

The progress of each patient was evaluated every two months on the basis of roentgenographic changes (parenchymal and cavitary) and of bacteriologic studies of sputum or gastric lavage.

The patients who received daily streptomycin tended to improve their disease more rapidly and more satisfactorily than did those on the thrice-weekly schedule, but this difference was not statistically significant. On the other hand, the daily dosage was accompanied by a significantly higher frequency of drug toxicity. Over-all, short-term observation of results *during* treatment seemed to reveal preference for the schedule of three injections weekly. The response to therapy was so closely similar in the two groups that it is presented for the entire series in table 1. Of 298 patients who still were on treatment at the eighth month, 55 per cent had improved markedly and 28.5 per cent moderately; at the twelfth month, two-thirds of 189 had improved markedly.

In almost one-half of the patients, thoracic collapse or excisional therapy was deemed advisable and was accomplished, for a total of 395 procedures, in 261 patients as follows:

Pneumonectomy	4
Lobectomy	37

[1] Hermann M. Biggs Memorial Hospital, Ithaca; Homer Folks Tuberculosis Hospital, Oneonta; Mount Morris Tuberculosis Hospital, Mount Morris; and Ray Brook State Tuberculosis Hospital, Ray Brook.

Segmental, block, or wedge resection	180
Thoracoplasty, primary	28
Thoracoplasty, other	74
Decortication	12
Phrenic nerve crush	6
Pneumoperitoneum	46
Pneumothorax	4
Other procedures	4

Chemotherapy was continued for eight or more months, rather than a briefer period, more often in the patients who also underwent collapse or excisional therapy than in those who did not. At each evaluation period, the percentage of "marked improvement" was higher in patients of the surgery-collapse group than in the others. Collapse or excisional therapy was elective; thus these results are descriptive rather than definitive.

The over-all rate of significant drug toxicity was 3.1 per 100 person-months of exposure,

TABLE 1

DEGREE OF IMPROVEMENT IN PATIENTS WITH PULMONARY TUBERCULOSIS *DURING* HOSPITALIZATION AND STREPTOMYCIN-PAS TREATMENT, BY EVALUATION PERIOD IN MONTHS

Evaluation Period*	Number of Patients	Degree of Improvement		
		Marked	Moderate	Slight or None
4	415	22.7	24.1	53.2
6	382	42.7	29.1	28.3
8	298	55.0	28.5	16.4
10	252	61.1	25.8	13.1
12	189	65.6	23.8	10.6
14	96	63.5	20.8	15.6
16	55	63.6	18.2	18.2

* In months after start of drug therapy

TABLE 2

BACTERIOLOGIC STUDIES OF SPUTUM OF PATIENTS WITH PULMONARY TUBERCULOSIS *DURING* HOSPITALIZATION AND STREPTOMYCIN-PAS TREATMENT, BY EVALUATION PERIODS IN MONTHS

Evaluation Period*	Number of Patients	Bacteriologic Studies of Sputum			
		Positive for *M. Tuberculosis*		Negative for *M. Tuberculosis*	
		Number	Per Cent	Number	Per Cent
4	363	105	28.9	258	71.1
6	322	35	10.9	287	89.1
8	246	18	7.3	228	92.7
10	204	10	4.9	194	95.1
12	151	8	5.3	143	94.7
14	73	5	6.8	68	93.2
16	39	4	10.3	35	89.7

* In months after start of drug therapy

which slightly exceeds that of some other reports. By advance agreement, critical criteria were applied in assessing slight evidences of toxicity and in resultant discontinuation of the drug. Each of the two drugs was responsible for approximately one-half of the toxic reactions. The rate of toxic reaction was highest during the first month of treatment, namely, 13.2 per 100 person-months of exposure; thereafter, and up to two years of treatment, it was less than 3 per 100 person-months. Reaction to streptomycin usually was manifested by vestibular nerve disorders; to PAS, by fever. At the four-month evaluation point, full bacteriologic studies (table 2) had been completed on 363 patients who were continuing treatment, and 105 still had tubercle bacilli in the sputum. In 15 of these patients, the strains of tubercle bacilli showed resistance to one, sometimes both, of the drugs employed. By the eighth month, infectiousness had been reversed in 228 of the 246 patients for whom full bacteriology had been reported. Among the 18 patients who continued to discharge tubercle bacilli in the sputum, only one patient-strain showed drug resistance.

There were 12 tuberculous deaths, a rate of 2.2 per 1,000 months of observation, and 8 deaths occurred from other causes.

DIVISION OF TUBERCULOSIS CONTROL
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April 15, 1955

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THE RELATIVE SENSITIVITY OF THE SKIN OF THE FOREARM AND SHOULDER TO TUBERCULIN

For many years it has been customary to carry out tuberculin and other tests for hypersensitivity by the intracutaneous injection of the diagnostic agent into the skin over the volar surface of the forearm. In the course of studies dealing with the tuberculin reaction following BCG vaccination, the present writers, as well as other investigators in this field, have long recognized the fact that the tuberculin reaction following BCG vaccination differs in most instances both quantitatively and qualitatively from that observed following natural infection with virulent tubercle bacilli.

In BCG-vaccinated persons, the tuberculin reaction tends to become less marked and more diffuse with passing years; and in many instances there may be a questionable, slight, or negative reaction to 0.1 mg. of a potent sample of Old Tuberculin (OT). It has further been observed that the vast majority of those persons who show a slight or doubtful reaction show an acute local inflammatory reaction (Koch phenomenon) when reinjected with BCG vaccine, indicating the persistence of specific hypersensitivity[1].

In the course of some studies dealing with the possibility of inducing a focal reaction by injecting OT in the vicinity of the BCG scar, it was found that, while focal reactions were not observed, the tuberculin reactions in the shoulder were more localized than the reactions noted in the volar surface of the forearm.

Further studies indicated that the more marked reaction to OT in the skin of the shoulder was unrelated to the BCG scar and, in all probability, was due to the more dense tissue in that region.

To determine this point, a group of 139 persons approximately twenty years of age, who had received an intracutaneous injection of 0.1 mg. of BCG vaccine from six to thirty months previously, were injected simultaneously with 0.1 mg. of freshly diluted OT in the skin of the forearm and in the skin in the region of the deltoid on the side opposite that of the BCG vaccination. Among the 139 persons, the forearm showed 92 with 1-plus, 2-plus, or 3-plus reactions; 43 were doubtful and 4 failed to react. On the other hand, readings at the site of the tuberculin injection over the deltoid showed 136 with 1-plus, 2-plus, or 3-plus reactions, 2 with doubtful reactions, and one who failed to react. With but few exceptions the edema and redness over the shoulder were more sharply defined and more marked than in the forearm.

These observations indicate that more satisfactory results are obtained when the tuberculin test following BCG vaccination is administered in the skin over the deltoid than in the volar region of the forearm.

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May 6, 1955

[1] ARONSON, J. D., AND MCGETTIGAN, M. T.: J. Immunol., 1951, *66*, 715.

THE CORRELATION OF RATE OF CATALASE ACTIVITY, GUINEA PIG VIRULENCE, AND ISONIAZID RESISTANCE OF TUBERCLE BACILLI FROM SPECIMENS OF PATIENTS UNDER ISONIAZID THERAPY

The significance of isoniazid-resistant tubercle bacilli in the sputum of patients receiving isoniazid therapy is not clearly understood at present. The frequent lack of correlation between drug-susceptibility tests, roentgenographic findings, and the clinical condition of such patients brings this fact into sharper focus. It is believed by some[1, 2] that the emergence of isoniazid-resistant tubercle bacilli in treated patients is an indication of a loss of drug effectiveness on the course of tuberculous infection. Others, taking into account these discrepancies, especially since the condition of some patients continues to improve in spite of the emergence of isoniazid-resistant tubercle bacilli in their sputum specimens, believe that too much attention is given to this phenomenon as determined by the semiquantitative cultural resistance tests[3].

At the present time, evidence is accumulating that certain properties of tubercle bacilli are changed when they have become isoniazid-resistant after exposure to the drug. For example, isoniazid-resistant tubercle bacilli manifest a loss of catalase activity *in vitro*[4] and a loss of virulence for guinea pigs[5].

The present report deals with the relationship of rate of catalase activity, degree of isoniazid resistance, and guinea pig virulence of tubercle bacilli from patients who have been receiving isoniazid treatment.

Forty-four sputum concentrates were used, and the following procedures were carried out on each concentrate:

Direct routine drug-susceptibility tests were performed on Peizer-Schechter agar and Löwenstein-Jensen slants. The tests consisted of the inoculation of approximately 0.25 ml. of each concentrate on duplicate medium slants containing serial dilutions of isoniazid. Control slants containing no drug were also inoculated with each concentrate.

At the same time, quantitative culture plate drug-susceptibility tests were carried out on each concentrated specimen of sputum. Duplicate Peizer-Schechter agar plates containing serial dilutions of isoniazid were inoculated with 0.1 ml. of concentrate which was evenly spread over the agar surface. Before the agar plates were seeded, the concentrated specimens were diluted with phosphate buffer at pH7. The amount of dilution was judged by Gaffky counts made on microscopy of stained specimens in order to ensure discrete colony growths for counting. From the colony counts and dilution factors it was possible to determine the total bacterial population on the control plates and the number of organisms resistant to each concentration of isoniazid.

Three-tenths (0.3) of a milliliter of each concentrate was injected subcutaneously into the groin of a guinea pig at the same time as the culture tests.

Catalase activity tests were carried out on the 44 two- to three-week-old growths of the primary control cultures of the tube and plate susceptibility tests.

The catalase test used in this study depends upon the action of hydrogen peroxide on a molybdate in acid solution[6]. The formation of various graded intensities of a yellow color, dependent upon the amount of hydrogen peroxide, permits a rapid colorimetric determination of the rate of catalase activity under controlled conditions. Two strains

[1] Coates, E. O., Jr., *et al.*: New England J. Med. 1953, *248*, 1081.
[2] British Medical Research Council Report: Brit. M. J., 1952, No. 4787, 735.
[3] Deuschle, K., *et al.*: Am. Rev. Tubere., 1954, *70*, 228.
[4] Middlebrook, G.: Am. Rev. Tubere., 1954, *69*, 471.
[5] Peizer, L. R., *et al.*: Am. Rev. Tubere., 1954, *70*, 728.
[6] Peizer, L. R., and Wideiock, D.: Am. Rev. Tubere., 1955, *71*, 305.

Table 1

Summary of Comparison of Isoniazid Resistance, Guinea Pig Virulence, and Catalase Activity of Tubercle Bacilli from 45 Sputum Specimens from Isoniazid-Treated Patients

Number	Specimen Number	Dilution Factor	Amount of Bacterial Growth From Concentrates on γ of Isoniazid per Ml. of Medium								Guinea Pig Virulence Results Within Three Months After Infection			Catalase Activity of Primary Cultures Grown Without Drug
			0(C)	0.1	1	5	10	25	50	100				
1	52										L	+	D	
	t.t.	—	4+	4+	3+	1+	1+	20 col.	15 col.	8 col.	Sp	+		
	plate	5	120	80	10	5	5	5	5	5	P	+		
2	46										L	+	D	
	t.t.	—	4+	4+	3+	1+	1+	50 col.	20 col.	0	Sp	+		
	plate	5	200	50	25	6	5	0	0	0	P	+	X	
3	79										L	+	D	
	t.t.	—	4+	1+	0	0	0	0	0	0	Sp	+		
	plate	5	200	25	0	0	0	0	0	0	P	+		
4	81										L	+	D	
	t.t.	—	4+	4+	4+	4+	5 col.	0	0	0	Sp	+		
	plate	10	300	150	30	25	0	0	0	0	P	+		
5	65										L	+	D	
	t.t.	—	4+	7 col.	8 col.	10 col.	8 col.	8 col.	6 col.	6 col.	Sp	+		100 per cent
	plate	—	150	20	20	5	7	5	5	0	P	+	X	
6	202										L	+	D	
	t.t.	—	4+	4+	4+	4+	0	0	0	0	Sp	+		
	plate	5	250	150	25	25	0	0	0	0	P	+	S	
7	201										L	+	D	
	t.t.	—	4+	4+	1+	1+	30 col.	0	0	0	Sp	+		
	plate	5	200	80	10	9	2	0	0	0	P	+		
8	218										L	+	D	
	t.t.	—	4+	4+	4+	3+	0	0	0	0	Sp	+		
	plate	10	100	250	50	30	0	0	0	0	P	+	X	Active
9	164										L	+	D	
	t.t.	—	4+	4+	4+	10 col.	0	0	0	0	Sp	+		
	plate	5	200	50	50	3	0	0	0	0	P	+	X	
10	207										L	+	D	
	t.t.	—	4+	4+	4+	4+	1+	0	0	0	Sp	+		
	plate	10	300+	100	50	30	8	0	0	0	P	+	X	
11	246										L	+	D	
	t.t.	—	4+	4+	2+	1+	0	0	0	0	Sp	+		
	plate	5	300	130	20	5	1	0	0	0	P	+	X	
12	245										L	+	D	
	t.t.	—	4+	4+	0	0	0	0	0	0	Sp	+		
	plate	10	300	50	0	0	0	0	0	0	P	+	X	
13	247										L	+	D	
	t.t.	—	4+	0	0	0	0	0	0	0	Sp	+		
	plate	10+	200	0	0	0	0	0	0	0	P	+		
14	273										L	+	D	
	t.t.	—	4+	4+	4+	1+	0	0	0	0	Sp	+		
	plate	5	300+	100	25	0	0	0	0	0	P	+		

(C) = control; t.t. = test tube; D = died; S = sacrificed; L = liver; Sp = spleen; P = point of inoculation; Col. = colonies; X = Culture from lesion susceptible to 0.1 γ per ml.

TABLE—I CONTINUED

Number	Specimen Number	Dilution Factor	Amount of Bacterial Growth From Concentrates on γ of Isoniazid per Ml. of Medium								Guinea Pig Virulence Results Within Three Months After Infection			Catalase Activity of Primary Cultures Grown Without Drug
			0 (C)	0.1	1	5	10	25	50	100				
15	264 t. t. plate	2	4+ 200	3+ 100	1+ 50	1+ 25	5 col. 0	0 0	0 0	0 0	L Sp P	+ + +	D	
16	167 t. t. plate	— 3	4+ 200	3+ 100	3+ 50	3+ 50	1+ 10	1+ 12	0 3	0 0	L Sp P	+ + +	D X	50 per cent
17	163 t. t. plate	— 5	4+ 250	4+ 150	4+ 150	4+ 50	1+ 20	1+ 5	1+ 1	1+ 2	L Sp P	+ + +	D X	
18	117 t. t. plate	— 3	4+ 250	4+ 200	4+ 200	2+ 50	3 col. 0	4 col. 0	4 col. 0	3 col. 0	L Sp P	+ + +	D X	Active
19	230 t. t. plate	10	4+ 200	4+ 150	4+ 30	3+ 30	2+ 0	0 0	0 0	0 0	L Sp P	+ + +	S	
20	229 t. t. plate	— 5	4+ 300	4+ 300	4+ 170	4+ 125	3+ 25	1+ 10	0 1	0 0	L Sp P	+ + +	S X	
21	203 t. t. plate	—	4+ 300	4+ 300	4+ 150	4+ 50	1+ 10	1+ 5	1+ 5	1+ 0	L Sp P	+ + +	S	
22	244 t. t. plate	— 2	4+ 250	4+ 200	3+ 200	1+ 100	0 5 col.	0 0	0 0	0 0	L Sp P	+ + +	D X	
23	89 t. t. plate	— 25	4+ 250	4+ 200	4+ 150	4+ 130	4+ 15	4+ 10	1+ 2	0 0	L Sp P	+ + +	D X	
24	49 t. t. plate	— 10	4+ 300+	4+ 280	4+ 200	4+ 150	4+ 30	1+ 5	10 col. 6	0 0	L Sp P	+ + +	D	
25	166 t. t. plate	— —	4+ 300+	4+ 300	4+ 200	4+ 150	2+ 30	1+ 20	1+ 25	1+ 20	L Sp P	— + +	S X	25 per cent
26	162 t. t. plate	— 10	4+ 250	4+ 250	4+ 150	3+ 30	2+ 15	1+ 1 col.	0 0	0 0	L Sp P	± + +	D X	
27	238 t. t. plate	— 5	4+ 300	4+ 250	4+ 150	2+ 50	10 col. 0	0 0	0 0	0 0	L Sp P	+ + +	D X	
28	133 t. t. plate	— 10	4+ 300+	4+ 300	4+ 200	4+ 100	4+ 50	4+ 50	4+ 20	4+ 20	L Sp P	— ± +	S X	Active
29	206 t. t. plate	— 2	4+ 300+	4+ 300	3+ 100	3+ 100	0 0	0 0	0 0	0 0	L Sp P	+ + +	D	
30	212 t. t. plate	— —	1+ 150	1+ 100	50 col. 80	0 0	0 0	0 0	0 0	0 0	L Sp P	± + +	D X	10 per cent Active

TABLE—1 CONTINUED

Number	Specimen Number	Dilution Factor	Amount of Bacterial Growth From Concentrates on γ of Isoniazid per Ml. of Medium								Guinea Pig Virulence Results Within Three Months After Infection			Catalase Activity of Primary Cultures Grown Without Drugs
			O(C)	0.1	1	5	10	25	50	100				
31	132										L	—	8	
	t.t.	—	4+	4+	4+	2+	1+	0	0	0	Sp	±		
	plate	—	300	200	200	150	30	1	0	0	P	+	X	
32	119										L	±	D	
	t.t.	—	4+	4+	4+	4+	4+	4+	4+	4+	Sp	+		
	plate	5	300+	250	150	150	125	25	25	25	P	+	X	
33	77										L	±	D	
	t.t.	—	4+	4+	4+	4+	4+	4+	4+	3+	Sp	+		
	plate	10	300+	300	200	100	75	30	30	20	P	+	X	
34	60										L	—	8	
	t.t.	—	4+	4+	4+	4+	4+	4+	4+	4+	Sp	+		
	plate	25	300+	250	175	50	25	25	2	Negative	P	+		
35	220										L	—	8	
	t.t.	—	4+	4+	4+	4+	0	0	0	0	Sp	+		10 per cent
	plate	10	250	220	220	100	0	0	0	0	P	+	X	
36	237										L	±	D	
	t.t.	—	4+	4+	2+	1+	1+	0	0	0	Sp	+		
	plate	—	250	200	100	30	25	0	0	0	P	+	X	
37	90										L	—	8	
	t.t.	—	4+	4+	4+	4+	3+	1+	30 col.	0	Sp	+		
	plate	10	300+	200	175	25	20	5	1	1	P	+	X	
38	72										L	±	8	
	t.t.	—	4+	4+	4+	2+	2+	0	0	0	Sp	+		Active
	plate	5	300+	250	250	50	10	0	0	0	P	+	X	
39	272										L	—	8	
	t.t.	—	4+	4+	4+	4+	2+	1+	0	0	Sp	—		
	plate	5	300+	300+	300+	300+	50	10	0	0	P	—		
40	270										L	—	8	
	t.t.	—	4+	4+	4+	1+	0	0	0	0	Sp	—		
	plate	5	250	250	250	50	0	0	0	0	P	±		
41	182										L	—	8	
	t.t.	—	4+	4+	4+	4+	4+	4+	4+	4+	Sp	—		
	plate	3	300+	300+	300+	300+	300+	100	100	50	P	±		
42	87										L	—	8	
	t.t.	—	4+	4+	4+	4+	4+	4+	4+	4+	Sp	—		
	plate	5	300	300	300	200	350	100	50	30	P	—		
43	68										L	—	8	Inactive
	t.t.		4+	4+	4+	4+	1+	0	0	0	Sp	—		
	plate	3	300	300	300	300	50				P	—		
44	57										L	—	8	
	t.t.	—	4+	4+	4+	4+	4+	4+	4+	4+	Sp	—		
	plate	25	200	200	200	200	200	50	25	5	P	±		

of tubercle bacilli known to be highly resistant to isoniazid and two known isoniazid-susceptible strains were always included as controls in each set of tests.

The test tube and quantitative plate culture isoniazid-susceptibility tests, guinea pig infection results, and the rate of catalase activity of tubercle bacilli of 44 sputum specimens are summarized in table 1. It may be observed that there was a good correlation between the guinea pig virulence results and the catalase-activity tests. When a primary culture of tubercle bacilli was either entirely or partially catalase-active, the corresponding animals had visceral tuberculous lesions at necropsy within three months after infection. Cultures prepared from the tuberculous lesions of 26 of the infected guinea pigs were all susceptible to 0.1 γ of isoniazid per ml. of medium. When the primary cultures of *M. tuberculosis* were catalase-inactive, there were no visible gross visceral tuberculous lesions seen in the corresponding infected guinea pigs (table 1, Nos. 39–44).

On the other hand, there seemed to be some, although a less definite, correlation between the results of the isoniazid-susceptibility tests and the rate of catalase activity of the primary cultures of tubercle bacilli.

The lack of a precise correlation between the drug-susceptibility tests and the catalase activity might have resulted either from a masking of the actual proportion of drug-susceptible tubercle bacilli in the mixed bacterial population in the specimens by the present methods used to test drug susceptibility; or from an erroneous interpretation of the concentration of isoniazid at which a population of tubercle bacilli might be considered entirely (not partially) resistant to the drug.

The first cause is easily demonstrated in table 1 by comparing the routine test tube isoniazid-susceptibility tests with the quantitative plate tests. The second reason, the erroneous interpretation of an isoniazid-resistant concentration, may be demonstrated in table 1, Nos. 39–44. It may be seen that the amount of growth of tubercle bacilli of No. 39 and No. 43 on 5 γ of isoniazid per ml. of medium equaled the growth on the controls containing no drug. These cultures were guinea pig-nonvirulent and catalase-inactive. No. 40 grew equally well on 1 γ of isoniazid per ml. of medium and on the control medium without any drug. Moreover, this culture was guinea pig-nonvirulent and catalase-inactive. Strains Nos. 41, 42, and 44 were resistant to 10 γ of drug per ml. of medium and grew as well on this concentration of isoniazid as on medium without any drug. These cultures were nonvirulent for guinea pigs and were catalase-inactive.

It is possible from the above evidence that different strains of tubercle bacilli may become isoniazid-resistant at different concentrations of isoniazid and that there is no one significant drug concentration at which all of the tubercle bacilli of a culture become resistant to isoniazid.

The degree of catalase activity of primary growths of tubercle bacilli from specimens may be one important clue to the actual extent of isoniazid resistance and guinea pig virulence of the organisms in specimens of drug-treated patients.

In summary: A comparison of the results of a quantitative plate study and a routine test tube method for the determination of isoniazid resistance of tubercle bacilli from specimens of drug-treated patients indicated that routine test tube procedures often mask the proportion of isoniazid-susceptible organisms in the mixed population of tubercle bacilli present in specimens. Guinea pigs infected with seemingly isoniazid-resistant tubercle bacilli developed typical tuberculous lesions within three months from which were grown tubercle bacilli susceptible to 0.1 γ of isoniazid per ml. of medium.

Good agreement seemed to exist between the rate of catalase activity of primary cultures of tubercle bacilli from isoniazid-treated patients and the guinea pig virulence of

these cultures. On the other hand, there seemed to be hardly any agreement of the isoniazid culture resistance results of these strains with either their catalase activity or their degree of guinea pig virulence.

No one significant isoniazid concentration was found at which cultures of tubercle bacilli became guinea pig nonvirulent and catalase-inactive.

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Lenore R. Pelzer
Daniel Wideloock

December 2, 1954

Correspondence

INDUCTION OF ARTIFICIAL PNEUMOTHORAX

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

My attention has been called to the Note by Clarke, Shipman, and Galbraith in the REVIEW (April, 1955) on "Induction of Artificial Pneumothorax" and to the reference in paragraph 2 (page 596) to "Dooneief, A. S., and Piccagli, R. W.: Am. Rev. Tubere., 1949, *60*, 557." The sentence preceding the bibliographical reference reads "The present writers take issue with the thesis that all initial pneumothoraces must be traumatic[5]." To most readers it would appear from this that the thesis that all initial pneumothoraces must be traumatic emanates from reference No. 5, i.e., Dooneief and Piccagli. In fact, however, the data in our paper to which the writers refer indicated the opposite and, on page 562 of the issue of the REVIEW referred to, we state: "... the theory that 'traumatic induction' is inevitable and unavoidable was not confirmed." The conclusion of our paper reads: "In the majority of patients with unilateral or bilateral moderately advanced pulmonary tuberculosis, the conventional method of pneumothorax induction with a blunt needle does not cause trauma to the lung of sufficient degree to permit escape of air demonstrable by available roentgenographic methods." This would appear to be exactly what the writers are saying in *their* paper.

The advances in chemotherapy and pulmonary surgery have made the point somewhat academic and rather unimportant. However, in the interest of scientific accuracy and journalistic precision, I thought that I ought to call this misquotation to the attention of the writers. Also, I wanted them to know that we are entirely in agreement.

Very truly yours,

ALFRED S. DOONEIEF

MONTEFIORE HOSPITAL
BEDFORD HILLS, NEW YORK

May 16, 1955

Books

MY LIFE WITH THE MICROBES. By Selman A. Waksman. Pp. 364, Simon and Schuster, New York, 1954, Price $5.00.

> Was not her name Fradia, which meant joy? Was not her husband's name Jacob, after one who was the father of her people? She would name her son, after her own father, whom she barely remembered, the King of Kings, Solomon the Wise, a name which had been corrupted during the centuries and had been changed to Zolmin.

Thus, in the year 1888, in the small town of Novaia-Priluka, twenty miles from the city of Vinnitsa in the Russian Ukraine, the infant Selman Abraham Waksman was born. Schooled in an atmosphere where first the prophets, then the Talmud with all its complicated interpretations, comprised most of the intellectual diet, he grew up during a disturbing period in the history of Russia. Poverty and dissatisfaction were prevalent among the peasants who had been liberated from slavery only a few decades before; socialism was growing rapidly, the Revolution was in the making. Thus, although there were no open rebellions against the government, some individuals felt the need for more opportunities in education and for greater participation in the life of the country.

Perhaps the most interesting aspect of this book is the author's account of life in the Ukraine during the early days of his youth, of the religious training acquired in his home, of his struggle for further education, and, finally, of the changes which led to his emigration to the United States in 1910. The many descriptive passages in which he relates his impression of Russia and of the Ukraine on his return there in 1924 and again in subsequent years clearly convey the economic and social changes that have taken place during the past half century. Written in Priluka, in 1924, he states:

> The greatest misery that one could ever imagine, the greatest catastrophe that the peoples in Russia have gone through; the greatest experiment in social and political relations of men can hardly express what we have seen. . . . Human life lost all vestige of value. A human being dressed in rags, living in a sometimes half-broken-down house, feeding on meat once a week. I have seen here, in the town where I was born and brought up, among the people whom I loved, misery as it could only be imagined. The first impression was as if I were walking through a cemetery, with a few living ghosts wandering among the ruins. This misery overshadows by far in my thoughts the greatest experiment in human relations ever attempted, namely the communistic regime in Russia."

By 1946, Priluka had been completely destroyed and all its inhabitants killed by the Germans.

From this rugged background comes a man who has survived to tell of the country of his birth, to describe to us his early impressions of the United States, and to write of close to half a century of fruitful research in the field of microbiology. In this autobiography, the author recounts numerous events which indicate his great contribution to the field of microbiology; he recounts, moreover, the sequence of events which initially aroused his interest in antibiotic substances and ultimately led to his discovery of streptomycin. Each step in his career, and each accomplishment, was a logical outgrowth of previous experiences; the discovery of so important a drug as streptomycin, by one interested primarily in soil microbiology, is not surprising, for, as the author reminds us: "The Lord created medicines out of the earth, and he that is wise shall not abhor them" (The Apocrypha—Ecclesiasticus).

Following the discovery of streptomycin in 1943, the author received honors from many nations and universities, chief among which was the award of the Nobel Prize for Physiology and Medicine. The new Institute of Microbiology at Rutgers University was built with royalties from the sale of streptomycin and assigned to that University by the author of this book. All of these, indicating the success achieved by one who followed a difficult course, are described in this book.

Due largely to the author's breadth of knowledge and wide contact with scientists throughout the world, due to his knowledge of the language and literature of the various countries of the world, and due also to his remarkable ability to depict in words his impressions of people, places, and things, this book contains much that will be of interest to laymen and scientists alike

GLADYS L. HOBBY
New York, New York

PRIMO-INFECTION ET RÉINFECTION DANS LA TUBERCULOSE PULMONAIRE: UNE ÉTUDE ANATOMIQUE ET PATHOGÉNIQUE BASÉE SUR 301 AUTOPSIES. By Georges Canetti. Pp. 261, Collection de L'Institut Pasteur, Editions Médicales Flammarion, Paris, 1954.

The author reports in this monograph the results of a study of the old remnants of the primary infection and of reinfection (either exogenous or endogenous) in 301 adult patients who died of progressive pulmonary tuberculosis in a hospital in Paris between 1940 and 1944. It appears from this study that pulmonary tuberculosis was predominantly of the nonprimary type and that latent reinfection foci were the more frequently found, the older the patients were. In most cases of reinfection the primary complex was calcified and, therefore, in the opinion of the author, could not have served as the source of bacilli involved in the "reinfection." This fact as well as the finding of an increasing number of reinfection foci in the older age groups suggest that the reinfection foci and the progressive disease itself were due to exogenous rather than endogenous tubercle bacilli. The author compares his results with those of similar investigations made in the United States by Terplan and by Medlar. The studies of these authors have shown that progressive pulmonary tuberculosis among American adults is a predominantly progressive primary infection. Canetti presents his evidence that these divergent results are explained by the difference of "density of tuberculous infection" in the two environments. He particularly emphasizes in this connection that the tuberculin conversion rate with age is much higher in France than in the United States as evidence for his thesis that the exposure frequency is much higher in France. In an environment in which the frequency of exposure is higher, not only the total number of cases of pulmonary tuberculosis but also the percentage of cases will be high in which pulmonary tuberculosis is due to reinfection, since the primary infection is followed by repeated exogenous reinfection. On the contrary, in an environment where the occasion for infection is rare, not only the total number of cases of pulmonary tuberculosis will be smaller but also the percentage of cases will be smaller where pulmonary tuberculosis is due to exogenous reinfection since the chance for such a reinfection is smaller. From the medical standpoint, progressive primary pulmonary tuberculosis is not different from the reinfection type. The important problem in the pathogenesis of both is that of determining what factors cause the evolution of a pre-existing focus either toward progression or toward healing. The author re-emphasizes that, in addition to systemic factors, local factors in the lesions themselves must play an important role in this evolution and that our knowledge concerning these factors is still deficient. Some insight of practical and theoretical importance into the problem presented by latent tu-

berculous foci might be obtained, the author suggests, if it were known whether the incidence of activation of such latent lesions could be reduced by chemotherapy, surgery, or bed rest alone or in combination; and he outlines in some detail how such a study could be conducted.

Canetti's critical anatomical investigations, integrated with those of Terplan and Medlar, present a well-rounded view of the complicated problem of endogenous versus exogenous reinfection tuberculosis. Canetti in this monograph has synthesized the clearest picture thus far presented of this aspect of the pathogenesis of tuberculosis.

WERNER B. SCHAEFER
Denver, Colorado

SURGERY IN TUBERCULOSIS. By Richard H. Overholt and Norman J. Wilson. Pp. 20, National Tuberculosis Association, 1955, Price, 50 cents.

This pamphlet is intended to explain in outline form the various operative procedures that have been employed in the treatment of pulmonary tuberculosis. It is intended primarily for medical students and nurses and those physicians who have had relatively little contact with pulmonary surgery. In simple outline form, with good diagrams, it indicates the principles of the various operative procedures and the anatomical changes which they produce. Naturally, in an outline of this type, the reasons for selecting a specific operative procedure cannot be indicated except in a very elementary way, but the pamphlet gives a clear and simple outline of the various surgical procedures that have been employed in pulmonary tuberculosis. The pamphlet fulfills the purpose for which it is intended.

HERBERT C. MAIER
New York, New York

BOOKS RECEIVED

ANTIBIOTICS AND ANTIBIOTIC THERAPY. By Allen E. Hussar and Howard L. Holley. Pp. 475, The Macmillan Company, New York, 1954, Price, $6.00.

AIRBORNE CONTAGION AND AIR HYGIENE. By William Firth Wells. Pp. 423, The Commonwealth Fund, Harvard University Press, Cambridge, Massachusetts, 1955, Price, $6.00.

UNTERSUCHUNG UND BEURTEILUNG DES HERZKRANKEN. By H. W. Knipping, W. Bolt, H. Valentin, and H. Venrath. Pp. 461, with illustrations, Ferdinand Enke Verlag, Stuttgart, 1955.

PROLONGED AND PERPLEXING FEVERS. By Chester S. Keefer and Samuel E. Lcard. Pp. 248, Little, Brown & Company, Boston, Massachusetts, 1955, Price, $5.50.

NOTICES

ANNUAL SYMPOSIUM ON PULMONARY DISEASES AT FITZSIMONS ARMY HOSPITAL

The Eighth Annual Symposium on Pulmonary Diseases will be held at Fitzsimons Army Hospital, Denver, Colorado, September 26–30 under the sponsorship of the hospital, the University of Colorado School of Medicine, and the American Trudeau Society. Topics such as physiological considerations and diagnosis, the management of pulmonary tuberculosis, complications of tuberculosis, chronic nontuberculous diseases, neoplastic and mycotic lesions, chronic suppurative diseases and other nontuberculous diseases, thoracic trauma and other surgical procedures and problems will be discussed. The symposium will also include medical and surgical chest conferences, case problems in a pulmonary disease clinic, and demonstrations conducted at the hospital. Registration for the course will be limited to 300 physicians in private practice, government service, and the armed forces.

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*Cannon, P. R.; Frazier, L. E., and Hughes, R. H.: Factors Influencing Amino Acid Utilization in Tissue Protein Synthesis, in Symposium on Protein Metabolism, New York, The National Vitamin Foundation, Inc., 1954, pp. 55-90.

The nutritional statements made in this advertisement have been reviewed and found consistent with current medical opinion by the Council on Foods and Nutrition of the American Medical Association.

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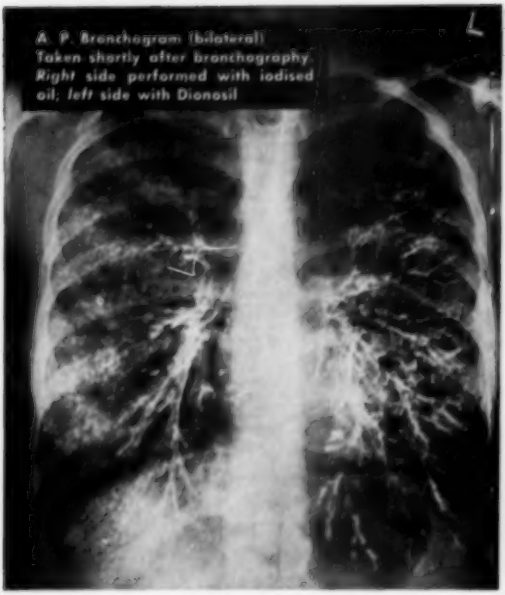
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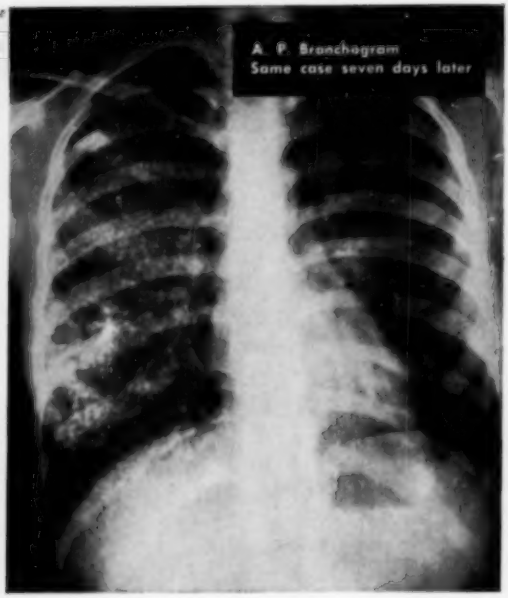

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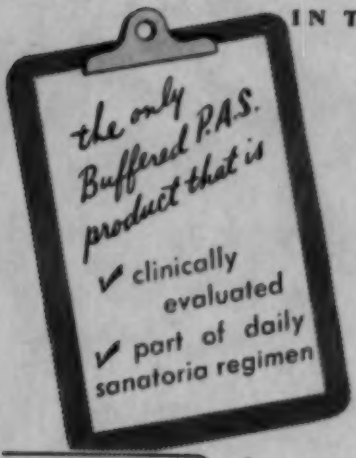

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References:

(1) *V.A. Quarterly Prog. Report, April '53 Bronx V.A. Hosp. N. Y.*

(2) *V.A. Quarterly Prog. Report, Oct. '54 Rutland Heights V.A. Hosp. Mass.*

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